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J. clin. Path. (1948), **1**, 197.

GIANT-CELL OR TEMPORAL ARTERITIS: A REVIEW

BY

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Historical

The first case of what is now known as temporal, cranial, or giant-cell arteritis was published in 1890 by Jonathan Hutchinson. He described how the 80-year-old father of a London Hospital beadle came to him complaining that he could not put his hat on because of painful swellings in his temples. These proved to be red, inflamed, swollen temporal arteries which subsequently lost their pulsations and were left as hard cords after the condition healed. The man recovered and lived for a number of years. The next case was recorded in 1930 by Schmidt. His patient showed the characteristic clinical picture and is of interest because he developed an intracranial aneurysm, a complication which has since been recorded in one other case (Andersen, 1947a). In 1932, Horton, Magath and Brown, of the Mayo Clinic, described two cases and, being presumably unaware of Hutchinson's and Schmidt's cases, regarded them as examples of a new disease and gave the condition the name of "temporal arteritis." They believed that the disease was limited to these arteries and followed a benign course free from complications. In 1934 and 1937 they recorded five further cases. In 1934 Paviot and others recorded the first case from France, but they did not mention any previously published cases and do not appear to have recognized the significance of their case. It is, however, interesting to note that their patient showed signs of involvement of the carotids as well as of the temporal arteries. In 1935 Barnard described the post mortem findings in a woman who had shown some of the signs of temporal arteritis and who had become blind. The temporal arteries were not described either in the clinical or autopsy account, but the description of the histology in the carotid and coronary arteries strongly suggests that this was an example of what is now called "giant-cell arteritis." In 1938 Jennings described the first two British cases corresponding to the descriptions of Horton and others, and he was the first to recognize blindness as a complication of this disease. Since then cases have been recorded from U.S.A., Britain, France, and Scandinavia; the total of published cases to date is 75.

Nomenclature

This disease was first called "temporal arteritis" by Horton and others in the belief that it was localized in these arteries. As further cases were recorded it became apparent that this was far from true. Kilbourne and Wolff (1946), being impressed by the frequency of involvement of the cerebral and retinal vessels, suggested the term "cranial arteritis," and this was adopted by Curtis (1946) and Meyers and Lord (1948). A few writers have included the eponym "Horton's disease," but this has fortunately not been followed. Gilmour (1941) introduced the title "giant-cell arteritis" and this was followed by Robertson (1947).

None of these titles is satisfactory. The disease is far too widespread in its distribution for either "temporal" or "cranial" to be accurate, and giant cells have not been demonstrated in all cases. Nevertheless, on the score of simple accuracy, "giant-cell arteritis" is certainly the best title of the three. There is, however, one point in favour of retaining Horton's original term, and that is that it draws attention to the dominating and most diagnostic clinical lesion. It seems likely that the terms "temporal" and "giant-cell" will flourish side by side.

Clinical Characteristics

(For summary of findings in the reported cases see Table.)

Sex.—The early reports included a high proportion of women, but since then more cases have been reported in men, and the figures now are 35 men to 40 women, which suggests that there is probably no real sex bias.

Age.—Giant-cell arteritis is a disease of the elderly, the mean age of the 75 recorded cases being 65.3 years. The majority have been over 60,

and all but two over 50. The exceptions were women of 22 (Meyers and Lord, 1948) and 23 (Gilmour, 1941). Gilmour's case was clinically atypical, but the histology of the vessels appears to be characteristic; the case of Meyers and Lord was a typical one clinically, though the biopsy, taken during a recurrent attack, showed only a healed arterial lesion.

Onset.—The disease begins as a vague illness, with malaise, anorexia, bodily aches, sweats, and often fever. This prodromal stage may last from a few days (Bain, 1938 ; Dick and Freeman, 1940) up to eighteen months (Cooke and others, 1946; Andersen, 1947), but usually lasts about one or two months. At the end of this time the patient develops pain in the head. This is usually referred to the temple, and is severe, often enough to interfere with sleep. In many cases it is described as coming in attacks of great violence with a dull ache between the attacks. The pain usually resists ordinary analgesics and may not be fully controlled by morphia. Soon after the onset of pains in the head the temporal arteries become extremely tender; they usually appear as tortuous nodular cords covered by a red, inflamed skin. In the early stages pulsation is still palpable but this is usually lost during the height of the disease, though it may return later (Profant, 1944). Many of the clinical reports refer to the arteries as thrombosed; but thrombosis is unusual in the descriptions of the histology, and the hard, cord-like texture is probably due to a filling of the lumen by intimal proliferation rather than by clot. Enlarged cervical glands have been noted by MacDonald and Moser, 1937, Dick and Freeman, 1940, Hoyt and others, 1941, and Scott and Maxwell, 1941. The associated clinical findings are usually few. The patients are ill out of proportion to the physical signs; they may show mental changes (Sprague and MacKenzie, 1940 ; Schaefer and Sanders, 1942 ; Broch and Ytrehus, 1946), but examination of the central nervous system usually reveals no physical signs or only equivocal and transitory ones. The blood pressure in these patients is most often normal, and the number of cases with hypertension is probably no greater than would be expected in a control population of this age. Similarly the signs of generalized vascular sclerosis are no greater than is likely in a random group of elderly people.

Laboratory findings.—Almost every known laboratory examination has been performed on these patients, and particularly tests for bacterial agglutination. These, and also the Wassermann reaction, have been consistently negative. Blood examination alone has given any reasonably constant results. Anaemia of hypochromic type (60 to 85 per cent haemoglobin) has been recorded in twenty-nine cases ; it is rarely severe. A leucocyte count has been recorded in thirty-six cases and has varied from normal up to 24,500 per c.mm. of blood (Profant, 1944), the mean figure being 10,500 per c.mm. The blood sedimentation rate has been recorded as raised in twenty-one cases, and whilst the variety of methods used precludes any mean figure, the results recorded have mostly indicated a very abnormal figure. This raised sedimentation rate persists throughout the course of the clinical illness, and may continue for some time after symptomatic recovery. The cerebrospinal fluid has been examined in a number of cases and has sometimes shown a rise of protein and cells, but these findings appear to be inconsistent. Blood cultures have been consistently negative, as have electrocardiograms and radiographs.

Course.—The disease runs a slow course over many months. In the sixty-two cases in which the duration is stated, it has varied from about eight weeks to thirty months (Cooke and others, 1946). The mean for the stated figures is 7.2 months, but this is almost certainly an underestimate because in a significant number of cases the patient had not fully recovered at the end of the stated time. As a rule the severity of the symptoms diminishes and the patient is discharged from hospital without major symptoms but often still far from fit, and final recovery to normal health may be delayed for a number of months.

Prognosis.—In their early papers Horton and others stated that the disease ran a benign course free from mortality and complications. This has since proved to be incorrect. Of the seventy-five recorded cases, fifty-nine have recovered and sixteen have died ; but it is difficult to assess how many of these died as a result of the disease. This will be discussed later.

Treatment.—No effective form of therapy has yet been discovered. Individual cases have responded to the most diverse forms of treatment. The case of Paviot and others (1934) responded dramatically to salicylates, and those of Justin-Besançon and others, and of Lucien and others responded to sulphonamides; but many other cases have failed to respond either to sulphonamides or penicillin. The one form of therapy which has given the most constant symptomatic relief has been biopsy of the temporal artery. This had been recorded in twenty-one cases. In only two cases has it been specifically stated that biopsy failed to

afford relief (Sprague and MacKenzie, 1940; Dantes, 1946). The relief is symptomatic, does not seem to affect the course of the disease, and may be due to the coincidental cutting of perivascular nerves. Such nerves have been noted in the fibrosed adventitia by Lucien and others (1939) and by the present author.

Complications.—The commonest and most serious complication is involvement of the eyes. This had been noted in twenty-five out of seventy-five cases, and in fifteen of these there has been permanent blindness of one (eight cases) or both eyes (seven cases). In the majority of cases the ophthalmoscopic findings have been trivial compared with the loss of function, and it is generally believed that the blindness is due to retinal ischaemia from involvement of the vessels behind the globe and out of range of ophthalmoscopic examination. Johnson and others (1943) suggested that this may be due to vascular continuity between the temporal and ophthalmic arteries via the lachrymal branch of the former, but since we now know that the disease is, in fact, widespread and involves many vessels, it is unnecessary to postulate vascular continuity.

The second most important complication is cerebral involvement. The cases of Sprague and MacKenzie (1940), Schaefer and Sanders (1942), and Broch and Ytrehus (Case 3, 1946) showed clinical evidence of cerebral damage but recovered. The cases of Gilmour (Cases 2 and 3, 1941) and Cooke and others (Cases 3 and 6, 1946) died of cerebral ischaemia, confirmed at autopsy. The cases of Chasnoff and Vorzimer (1944), Cooke and others (Case 1, 1946), and Curtis (1946) died from cerebral causes but autopsy was not performed. In addition, the cases of Schmidt (1930) and Andersen (1947a) developed signs of intracranial aneurysm. From the records of the cases which came to autopsy there is little doubt that the cerebral signs and symptoms are due to involvement of the cranial vessels and are due to ischaemia. They may be transitory or they may be fatal, but generally they do not seem to leave any sequelae if the patient recovers; though there seems to be no reason why this should not occur. The two cases of Sjövall and Windblad (1944) both developed a crippling arthritis, but they are the only two recorded cases to do so and it is possible that they represent a coincidence rather than a complication. Robertson's (1947) first case developed gangrene of the leg, but again that may have been a coincidence due to atheroma. It is certainly significant that, in a disease which causes gross vascular narrowing and affects medium-sized arteries, there is no ischaemic gangrene of the limbs.

Fatal cases and the extent of the disease.—The cases which have died are of sufficient interest to justify individual consideration. The first was that of Barnard (1935). This woman had been ill for seven months with an apparently undiagnosed condition which was characterized by headache and the loss of sight in one eye. She died of erysipelas, and at routine autopsy changes were noted in the internal carotid and coronary arteries. These lesions were characterized by medial necrosis and cellular infiltration, including giant cells, and were interpreted as tuberculous though no bacilli were found. In the light of the numerous cases since recorded it seems highly probable that this was an example of giant-cell arteritis in which the involvement of the temporal vessels was minimal. A similar case has been recorded by Andersen (1947b).

Sproul and Hawthorne (1937) recorded two cases neither of whom showed any significant signs of temporal arteritis and both of whom died, one of septic kidney and the other of cardiac infarction. One case showed histological lesions in the aorta and iliac artery and the other in the aorta, iliac, and carotid arteries. The descriptions strongly suggest that in these cases the arterial lesion was indistinguishable from that of giant-cell arteritis, though the temporal arteries were not apparently involved clinically and were not examined at autopsy. The authors did not regard their cases as being examples of giant-cell arteritis, and Sproul does not refer to this publication in a later paper (1942), in which he records the autopsy findings in a classical case of giant-cell arteritis who died of ischaemic heart disease. In the latter case there were typical and severe lesions in the aorta, innominate, subclavian, pulmonary, coeliac, mesenteric, renal, and iliac arteries (the temporal arteries were not examined at autopsy though they were clinically typically affected during life).

Gilmour (1941) recorded four autopsy cases. The first was of a woman of only 23 who had been ill for six months with giddiness and hot sweats after an influenza-like onset and who died of a ruptured subclavian aneurysm. Clinically the case was atypical, but the histology of the aorta, internal, and external carotid and subclavian arteries was exactly similar to the published descriptions of classical cases and it seems probable that this was a true case. The age is quite unusual, but a clinically typical case has been reported in a woman of 22, by Meyers and Lord (1948). Gilmour's second case, in a woman of 59,

TABLE

RECORD OF PUBLISHED CASES OF GIANT-CELL ARTERITIS

No.	Authors	Case Nos.	Year	Sex	Age	Duration	Recovered or died	Biopsy	Autopsy	Eye symptoms or signs	Fever	Illness	B.S.R.	W.B.C.	Bodily pains	Anaemia	Vessels affected	Relieved by biopsy	Comments
1	Hutchinson		1890	M	80	?	R	—	—	—	—	—			—		Both temp.		
2	Schmidt		1930	M	69	8/12	R	—	—	Scotoma	+	+		3,900	?	+	Both temp.		Intracranial aneurysm
3	Horton *et al.*	1	1932	F	55	22/12	R	+	—	—	+	+		13,700			Both temp.		
4	„ „ „	2	1932	M	68	2/12	R	+	—	—	+	+		10,600			Both temp.		
5	Paviot *et al.*		1934	M	61	3/12	R	—	—	Unequal pupils	+	+			+		Both temp., both carotid.		Associated with active rheumatism.
6	Barnard		1935	F	63	7/12	D	—	+	L. blind	—	+		8,600		+	Carotid and coron. Both temp.	+	Glands +. Root abscess.
7	MacDonald and Moser		1937	F	60	6/12	R	+	—	—	+	+					Both temp.		
8	Horton and Magath ..	1	1937	F	69		R	+	—	—	+	+					Both temp.		
9	„ „ ..	2	1937	F	75		R	—	—	—	+	+					Both temp.		
10	„ „ ..	3	1937	M	72		R	+	—	—	+	+					Both temp.		
11	„ „ ..	4	1937	M	65		R	—	—	—	+	+					Both temp.		
12	„ „ ..	5	1937	F	68	6/12	R	+	—	—	—	+					Both temp.		
13	„ „ ..	6	1937	M	57	2/12	R	—	—	—	+	+					Aorta and iliac.		Died of septic kidney.
14	Sprout and Hawthorne..	1	1937	M	76		D	—	+	—	—	—					Aorta, iliac, caro-		Died of cardiac infarct.
15	„ „ ..	2	1937	M	50		D	—	+	—	—	—					tid.		
16	Jennings	1	1938	F	66	6/12	R	+	—	L. blind	+	+	+	13,000	+	+	Both temp.		
17	„	2	1938	F	72		R	—	—	—	+	+		9,100		+	Both temp.		
18	Bain		1938	F	71	10/52	R	—	—	—	+	+		12,500			Both temp.		
19	Thévenard		1939	M	66	9/12+	R	+	—	—		+					Both temp.	+	
20	Lucien *et al.*		1939	F	79		R	+	—	—	+	+		3,500			Both temp. and occipital.		
21	Dick and Freeman ..	1	1940	F	65	3/12	R	+	—	L. reduced field	+	+		13,100			Temp., pariet., and P. auric.		Glands +. Followed pneumonia.
22	„ „	2	1940	F	76	13/12	R	—	—	Blurred vision	+	+	N		+		Both temp.		Glands +. Tonsils inflamed.
23	Bowers		1940	F	65	6/12	R	+	—	—	+	+	+			+	Temp. and occipital	+	
24	Sprague and MacKenzie		1940	M	66	6/12+	R	+	—	—	+	+	+	12,900		+	Both temp.	—	Became mentally sluggish.
25	Hines		1941	F	69	6/12	R	+	—	—	+	+				+	R. temp. and radial.		
26	Hoyt *et al.*	1	1941	F	58	7/52+	R	+	—	—	+	+	+	20,000	+		Both temp.	+	Glands +. Inflamed fauces.
27	„ „ „	2	1941	F	61	7/52+	R	+	—	—	+	+		14,000	+		Both temp.	+	Glands +.
28	„ „ „	3	1941	M	67	3/12+	R	+	—	—	—	+			+		Both temp.		
29	Scott and Maxwell ..		1941	F	70	6/12+	R	+	—	L. blind	+	+	+	10,300	+	+	Temp., facial, and carotid.	+	Glands +.
30	Gilmour	1	1941	F	23	6/12	D	—	+	—	—	+					Aorta, carotid, and subcl.		Died of ruptured subclavian aneurysm.
31	„	2	1941	F	59	5/12	D	—	+	—	?	+					Aorta, carotid, cerebral.		Cerebral infarct.
32	„	3	1941	M	63	9/12	D	—	+	Failing vision	—	+			+		Aorta and carotid.		Cerebral infarct.
33	„	4	1941	F	64	26/12	D	—	+	—	—	+		4,000	—	+	Aorta, innom., subcl., carotid, and iliac.		Post-operative death. Healed arteritis.
34	Sproul		1942	M	68	8/52	D	—	+	—	—	+	+	17,000	—	—	Temp. and others.		Many major vessels affected.
35	Plaut		1942	F	63		R	+	—	—	+	+			+		Both temp.		
36	Schaefer and Sanders ..		1942	F	62	10/52	R	—	—	—	+	+		9,700	—	—	R. temp.		Delirious. C.S.F. changes
37	Oldberg		1942	M	64	3/12	R	—	—	—	+	+	+			+	Both temp.		
38	Murphy		1942	F	71	6/12	R	+	—	Ptosis							Both temp.		
39	Johnson *et al.*	1	1943	F	61	6/12	R	+	—	L. blind	+	+					Both temp.		
40	„ „ „	2	1943	M	75	3/52+	R	—	—	Both blind							Both temp.		
41	„ „ „ ..	3	1943	F	80		R	—	—	One blind		+					Temp.		
42	„ „ „	4	1943	F	76	4/12+	R	—	—	R. blind							Both temp.		

TABLE—*continued.*

No.	Authors	Case Nos.	Year	Sex	Age	Duration	Recovered or died	Biopsy	Autopsy	Eye symptoms or signs	Fever	Illness	B.S.R.	W.B.C.	Bodily pains	Anaemia	Vessels affected	Relieved by biopsy	Comments
43	Brown and Hampson ..		1944	M	61	6/12+	R	+	—	—	—	+			+		Temp. and occipital.		
44	Chasnoff and Vorzimer		1944	F	63	14/12	D	+	+	—	+	+	+	9,200	+	+	Temp. and internal.	+	Died in coma 3/12 after biopsy. P.M. details not published.
45	Profant	1	1944	F	76	12/12	R	—	—	—	+	+		24,500	—	—	R. temp.		Flare up after tooth extn.
46	,, ...	2	1944	F	61	2/12+	R	—	—	—	+	+		12,000			Both temp.		Pulsations reappeared.
47	Sjövall and Windblad ..	1	1944	M	62	12/12	R	+	—	—	+	+	+	5,900		+	Both temp.	+	Developed arthritis.
48	,, ,, ..	2	1944	M	63	9/12	R	+	—	—	+	+	+	7,300	+	+	Both temp.		Developed arthritis.
49	Shannon and Solomon..		1945	M	73	12/52	R	+	—	Both blind							Temp. and ? occipital.	+	
50	Cooke *et al.*	1	1946	M	66	5/12	D	+	—	L. blind	+	+		11,800	+	—	Temp. and ? limb.	+	Died cerebral death 6/52 after discharge. No P.M.
51	,, ,, ,, ...	2	1946	F	66	6/12+	R	—	—	Both blind	+	+		10,700	+	+	Both temp.		
52	,, ,, ,, ...	3	1946	F	69	30/12	D	+	+	Both blind	—	+		5,100	+	+	Both temp., aorta, and femoral.		Died of cerebral damage.
53	,, ,, ,, ...	4	1946	M	71	6/12+	R	+	—	—	—			8,400		+	Both temp.	+	
54	,, ,, ,, ...	5	1946	M	69	12/12+	R	+	—	Vision impaired	+	+		10,000	+		Temp. and occipital.	+	Had second attack. ? limb arteries involved.
55	,, ,, ,, ...	6	1946	F	73	14/12	D	+	+	Both blind	—	+		8,300	+	+	Temp. and radial, and others.	+	Cerebral death. Many int. arteries affected.
56	,, ,, ,, ...	7	1946	F	68	12/12+	R	+	—	—	+	+		13,200	+	+	Temp. and occipital.	+	
57	Broch and Ytrehus ..	1	1946	M	69	5/12	D	—	—	Squint	+	+	+			+	Both temp.		Died 2/12 later of haematemesis. No P.M.
58	,, ,, ..	2	1946	M	76	12/12	R	—	—	—	—	—	+			+	Both temp.		
59	,, ,, ..	3	1946	F	67	6/12	R	—	—	—	+		+			+	Both temp.		
60	,, ,, ..	4	1946	M	74	12/12	R	+	—	—	+	+	+			+	Both temp.		Mental changes.
61	Curtis		1946	F	71	4/12	D	+	—	Both blind	+				+		Both temp.		Cerebral death. No P.M.
62	Dantes		1946	F	55	6/12+	R	+	—	—	+						Temp. and leg.	—	Dorsalis pedis involved.
63	Gordon and Thurber ..		1946	M	65	19/52	R	+	—	—	+				+	+	Temp. and limb.		
64	Kilbourne and Wolff ..		1946	M	68	3/12	D	+	—	Retinal exudate	+	+		13,600	—	—	Both temp.		Died of cardiac infarct 3/12 later. No P.M.
65	Andersen		1947a	M	54	24/12	R	+	—	Glaucoma	+	+	+	7,300	+	+	Both temp.		Left with limb pains and intracranial aneurysm.
66	Andersen		1947b	F	63	5/12	R	+	—	—	+	+	+	4,700	+	+	Temp. and leg.		Temp. arteries less affected than others.
67	Robertson	1	1947	M	65	12/12	R	+	—	—	+	+	+	6,600	+	+	Temp., facial, occip., and leg.	+	Leg gangrene, ? cause.
68	,, ...	2	1947	M	65	2/12+	R	+	—	Both blind	+	+	+	9,000	+	+	Temp., facial, and occip.	+	
69	,, ...	3	1947	F	73	?	R	—	—	—	+	+	+				Temp., facial, and occip.		
70	,, ...	4	1947	M	67	?	D	—	+	—		+			+		Temp. and occipital.		Died P.G.U. No P.M. histology. Thrombus in subclavian.
71	Justin-Besançon *et al.* ..		1948	F	70	4/12	R	+	—	—	+	+	+	10,500	+	+	Temp., occip., and facial.	+	? similar attack 3 years before.
72	Meyers and Lord ..		1948	F	22	3/12	R	+	—	—	+	+					L. temp.	+	Biopsy in recurrent attack, healing lesion.
73	Cohen and Harrison ..	1	1948	M	72	15/52	R	+	—	—	—						Both temp.	+	
74	,, ,, ...	2	1948	M	65	8/52	D	+	—	—	+	+		12,800		+	Both temp.	+	Died 6/12 later from cardiac infarct. No P.M.
75	,, ,, ...	3	1948	M	69	23/52	R	+	—	L. blind	—		+	6,200		—	Both temp.	+	

had a five months' illness starting like influenza and characterized by temporal pain and noises in the head. She died in coma and at autopsy showed thrombosis of the internal carotid artery, and giant-cell arteritis of it and of the aorta. Gilmour's third case, a man of 63, had a nine months' illness with headache, mental signs, and failing vision. He too died in coma and at autopsy showed thrombosis of the internal carotid arteries and giant-cell arteritis of the aorta and of the external and internal carotid arteries. In Gilmour's fourth case, a woman of 64 suffered an ill-defined disease for twenty-six months and never showed any indication of temporal arteritis. She died of enteritis and amyloid kidneys, but at autopsy the aorta, innominate, common carotid, subclavian, and iliac arteries showed what the author interpreted as the healing stage of giant-cell arteritis. Chasnoff and Vorzimer (1944) record a case with typical histological appearances in the biopsy specimen of the temporal artery. Three months after discharge the patient returned to hospital in coma and died. The authors stated that at autopsy other internal arteries were involved and that details were to be published later by Mahoney and Hall, but no such publication can be traced.

Cooke and others (1946), in a series of seven typical cases of temporal arteritis, record three that died. Their first patient died from cerebral causes six months after discharge, and no autopsy was performed. Their second died from cerebral causes, and giant-cell arteritis was found in the aorta and in the femoral and mesenteric arteries. Their third also died from cerebral causes, and giant-cell arteritis was found in the aorta and in the superior mesenteric, femoral, radial, and retinal arteries. Broch and Ytrehus (quoted by Andersen, 1947a) record a typical case in which the patient died of haematemesis two months after discharge from hospital, but no autopsy was performed. Curtis (1946) described a patient with typical histological appearances in the biopsy specimen of the temporal artery who was readmitted in coma eighteen days after discharge and died, but no autopsy was carried out. Kilbourne and Wolff (1946) report a patient with typical biopsy findings who died of cardiac infarction three months later; no autopsy was carried out. Robertson (1947) recorded a clinically typical patient who died a month later of a perforated gastric ulcer. At autopsy the subclavian artery was thrombosed but, unfortunately, no sections were taken.

The inclusion of some of these sixteen cases is open to criticism on the ground that as they did not show the clinical signs of temporal arteritis during life, therefore the arterial disease found at autopsy is something different. Dantes (1946) has denied the authenticity of the cases of Barnard, Gilmour, and Sproul and Hawthorne. In the author's opinion this criticism is not justified for the following reasons. There is undeniable clinical and autopsy evidence that temporal arteritis is a widespread vascular disease. The clinical syndrome on which the diagnosis depends is the result of involvement of the superficial temporal arteries, and if these are excised for biopsy one of the dominant symptoms (localized head pain) usually disappears. If, therefore, a case of "temporal arteritis" should occur in which the temporal arteries were not involved the only really diagnostic clinical features would be lacking. In the cases recorded by Cooke and others, Sproul, and Chasnoff and Vorzimer the diagnosis was established clinically and was beyond question, and in these the larger internal arteries were involved. In the cases of Barnard, Gilmour, and Sproul and Hawthorne, the internal arteries showed apparently similar lesions, but the temporal arteries were not noted clinically or examined histologically and are, therefore, presumed not to have been involved. Nevertheless, it seems reasonable to assume that these were true cases and that in them the temporal arteries were either not involved or involved so slightly that they were overlooked.

These sixteen cases may give an exaggerated impression of the fatality rate in temporal arteritis. The cases of Barnard, Sproul and Hawthorne, and Gilmour (Case 4) did not die of their arterial disease. The cases of Sproul, Chasnoff and Vorzimer, Broch and Ytrehus, Curtis, Kilbourne and Wolff, and Robertson may have died of other diseases and not directly of the arteritis. Nevertheless, in the light of the first three cases of Gilmour and the second and third cases of Cooke and others, there is no doubt that temporal arteritis can prove fatal by cerebral involvement and it seems likely that it may also be fatal by involving other vital arteries.

These autopsy cases also illustrate the widespread nature of the disease, a point which has been stressed by clinical observers. Giant-cell arteritis is a disease of the larger arteries. The characteristic lesions have been described microscopically in the aorta nine times, in the carotid arteries six times, in the iliac arteries four times, in the mesenteric and subclavian arteries three times, in the innominate and femoral arteries twice, and in the pulmonary, coronary, coeliac, renal, radial, and retinal arteries once each. Most of these are arteries which are readily accessible dur-

ing the course of a routine autopsy, and the implication is that the disease is likely to affect any of the elastic and larger muscular arteries. It seems highly probable that other, less accessible, arteries were affected in these cases but were not examined.

Another point which emerges from these autopsy reports is the irregular distribution of the lesions. In most cases a number of vessels other than those listed above were examined and found unaffected. A further point is that the disease is apparently limited to arteries; the only reference to veins is made by Cooke and others, who state that the femoral vein was involved in one of their autopsy cases. It is, of course, possible that minor grades of venous involvement might be found if veins were systematically examined, but it is unlikely that the disease affects them severely or the naked-eye changes would have been noted. Apart from the larger arteries noted at autopsy there is clinical evidence that other arteries than the temporal are involved, especially the palpable vessels of the head. The vessels noted clinically are as follows: occipital artery (Lucien and others; Bowers; Brown and Hampson; Shannon and Solomon; Cooke and others; Robertson; Justin-Besançon and others); facial artery (Scott and Maxwell; Robertson; Justin-Besançon and others); carotid artery (Paviot and others; Scott and Maxwell); parietal artery (Dick and Freeman); posterior auricular artery (Dick and Freeman); radial artery (Hines); dorsalis pedis (Dantes).

Pathology

The lesions in the temporal arteries.—The following account of the morphology of the lesions is based partly on the published reports and partly on a personal examination of five cases. The gross morbid anatomy is not characteristic. The vessels are bigger than normal and may be nodular. They appear to be solid cords with little or no lumen. Histologically the lesions are much more striking and characteristic (Plate Ia) but vary a good deal in their distribution. At different levels within one short segment of artery the lesion may vary in its intensity, in the presence or absence of the characteristic giant cells, and in the proportion of the circumference involved. In a suspected case, if the histology is atypical it is wise to cut deeper into the block. The lumen is usually reduced to a minute hole or slit and may be thrombosed, but thrombosis is the exception rather than the rule. It has been stated to be present thirteen times and absent thirty times, and probably represents no more than an incidental complication. It is certainly true that thrombosis plays no essential part in the development of the lesion—a significant point of differentiation from Bürger's disease. The intima shows gross thickening, thus accounting for the diminished lumen. This is invariably present and has been mentioned in every histological report. The intimal thickening may be either concentric or eccentric, usually depending on whether the whole circumference of the media or only a part of it is affected.

During the active stage of the disease this intimal proliferation is often divisible into two different zones (Plate V). The inner one, which is usually the thicker, consists of a cellular fibrous tissue of loose texture with relatively few inflammatory cells or vessels. In the interstices between the fibroblasts there is a pale-staining background which has been described by Sjövall and Windblad (1944) and Gordon and Thurber (1946) as oedema, and by Brown and Hampson (1944), and by Gilmour (1941) as mucoid. In the cases which I have examined this material has been of mucoid nature (Plate IVa) and apparently identical with the mucoid interstitial tissue described by Schultz (1922) and Ssolowjew (1924) in all arteries, and so constantly seen in excess in medionecrosis of the aorta. It seems probable that it represents a normal product of the connective-tissue cells of arteries.

In the outer part of the intima the picture is usually one of a much more active inflammatory response, but this is not always so; some arteries lack this zone entirely (Plate IIb). There are often young capillaries (Plate V) and nearly always an inflammatory cellular infiltration. The cell response varies from one case to another. Lymphocytes, macrophages, plasma cells, and polymorphonuclears may be present in variable proportions, and some giant cells may be seen. Eosinophils are rarely present and are practically never a marked feature—a point of distinction from polyarteritis nodosa. The media usually shows the maximum damage and is often virtually destroyed. Frank coagulative necrosis is frequently present (Plate V) and has been specifically mentioned in twenty-one reports. Cellular infiltration is always present and is rather variable. Lymphocytes and macrophages always appear to be present (Plate IIb and III), and there are usually some polymorphonuclears but they are rarely the dominant cell. Eosinophils are occasionally mentioned (Bowers; Hoyt and others; Kilbourne and Wolff) but never as a dominant cell.

The characteristic giant cells (Plate IIb and III) are mentioned as present in thirty-eight cases and can be inferred to have been present in most of the others. They were apparently not present in the

cases of Kilbourne and Wolff, Gilmour (Case 4), and Sjövall and Windblad (Case 2). I have seen a case in which no giant cell was present in the first few sections of a biopsy specimen but plenty were present deeper in the block, and it seems probable that all or nearly all cases will show giant cells if the specimen is cut at different levels. These giant cells are of foreign-body type and of irregular shape, sometimes rounded, sometimes elongated. They vary in size from cells of 10μ with two or three nuclei, up to masses of 100μ with very many nuclei. The latter may be distributed in any way, usually being quite haphazard, but sometimes arranged round the periphery exactly like the giant cells of a tubercle. The nuclei resemble those of the neighbouring macrophages, and the cytoplasm has an eosinophilic ground-glass appearance. Some of these giant cells lie in relation to fragments of elastic tissue (Plate IVb) and presumably represent an attempt at its removal, but others occur in the thickened intima in situations away from demonstrable elastica. The internal elastic lamina usually shows severe destruction (Plate IIb). This is recorded in twenty-eight cases and can be inferred from some of the other reports. It is generally fragmented into short lengths, and these may disappear. Sometimes the fragments assume odd shapes. In some cases there is a later overgrowth of new elastica (Cooke and others). These medial lesions are generally rather diffuse, involving large segments or the whole circumference, but in some cases they occur in a curiously focal form. This was so in Barnard's case, where they resembled tubercles, and is beautifully depicted in Fig. 4 of the paper by Cooke and others (1946).

The adventitia is involved to a variable extent (Plate IIa). The adventitial lesions were stressed by Horton and others in their original papers, but subsequent observers appear to have been more impressed by the medial changes. Cooke and others suggest that the lesion spreads along the vessels by the vasa vasorum in the adventitia. In the majority of cases there is a cellular infiltration of the adventitia, contiguous with and similar to that of the media but lacking giant cells. This is usually associated with an overgrowth of adventitia fibrous tissue. The vasa vasorum may be cuffed by inflammatory cells; this has been stressed by Gordon and Thurber and by Kilbourne and Wolff. In some cases nerves are visible embedded in the fibrosed adventitia (Lucien and others, 1939; Cohen and Harrison, 1948). Such involvement of nerves may account for the pain in the temporal regions and for its relief when a biopsy is performed.

Giant-cell arteritis in larger arteries.—In the cases which have come to autopsy the morphology of the lesions in the larger arteries has been essentially similar to that in the temporal arteries. Furthermore, the descriptions in the clinically uncertain cases of Barnard, Gilmour, and Sproul and Hawthorne tally exactly with those of the clinically proved cases of Cooke and others and of Sproul.

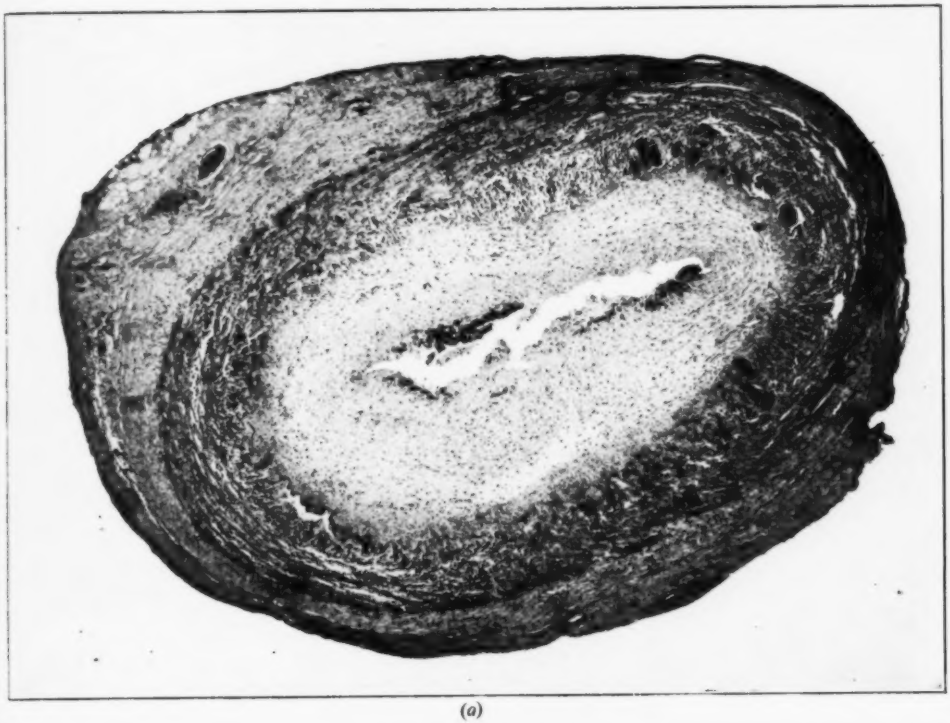
Macroscopically the vessels (aorta, carotid, etc.) may not show any obvious lesion or they may show a smear of fibrin clot overlying the lesion. Microscopically the damage affects mainly the media. The intima may show some recent fibroblastic proliferation, but this is slight and may be absent. Cellular infiltration of the intima is the exception rather than the rule. In the media the striking lesion is a cellular infiltration in which lymphocytes and plasma cells dominate, though a few polymorphonuclears may be seen. Giant cells are always present (Gilmour's Case 4 is an exception) and are of the same type as those in the temporal arteries. They may be found in association with fragments of elastica. Necrosis is usually only a cellular one, with disappearance of muscle in the affected areas, but occasionally foci of dead tissue may be seen. Some destruction of elastica is nearly always present, but it is slight and affects only few fibres. There may be increased vascularity of the media, but this is not striking. In the adventitia, changes are slight or absent. There may be some fibrosis, and there may be some cellular infiltration similar to that of the media.

In arteries intermediate between the common carotid and the temporal the lesions are also intermediate, and there is a greater tendency to thickening of the intima and to thrombosis.

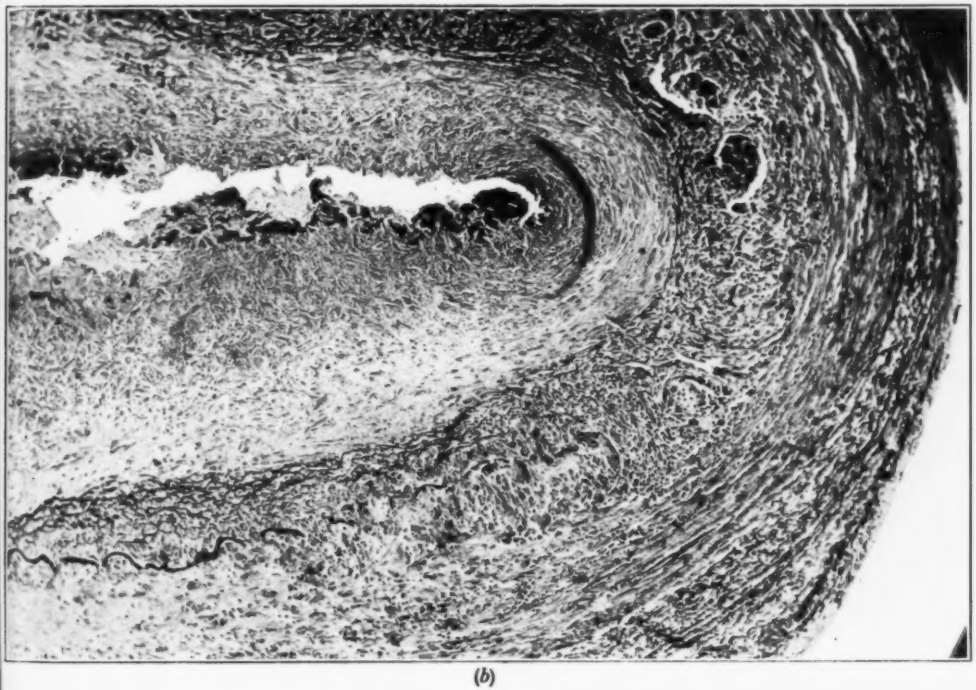
From a study of these cases there seems to be no reasonable doubt that the giant-cell arteritis observed in the larger arteries is the same as that in the temporal arteries and that the disease is widespread.

Aetiology

Nothing is known of the aetiology of giant-cell arteritis. There are, however, certain points which appear significant. It affects white races only; at least no cases have been recorded in coloured races. The early cases (Horton and others) were mostly in farmers and country dwellers, but this has not been the rule in subsequent cases. On the whole, middle-class and well-to-do patients seem to have been rather more frequently affected, but no occupation has been unduly represented. There is no evidence of a familial incidence. No organisms have been recovered with any regularity from

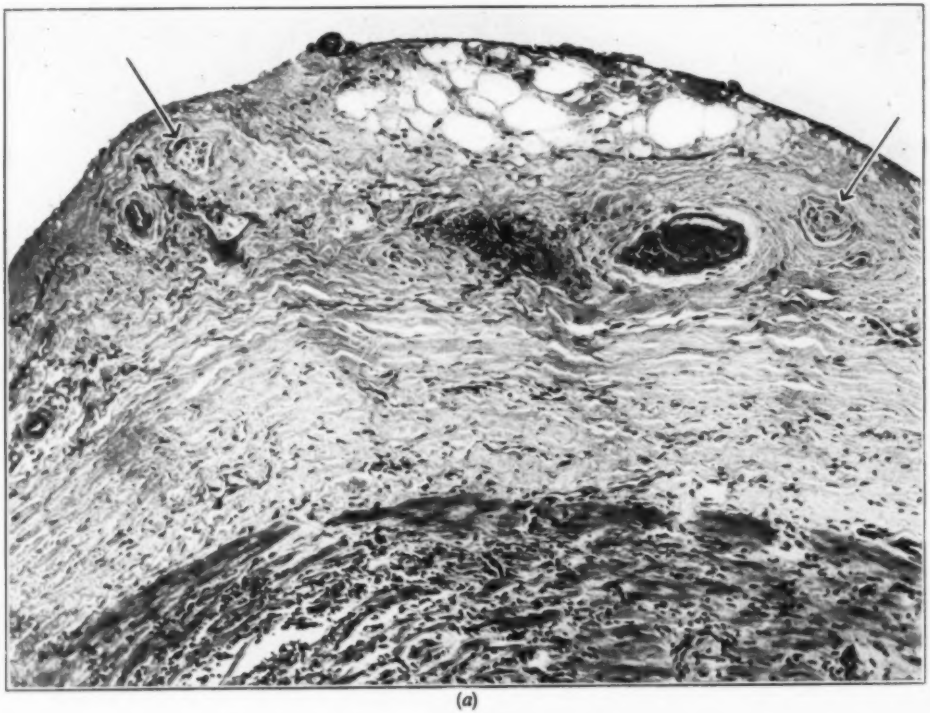


(*a*)



(*b*)

PLATE I.



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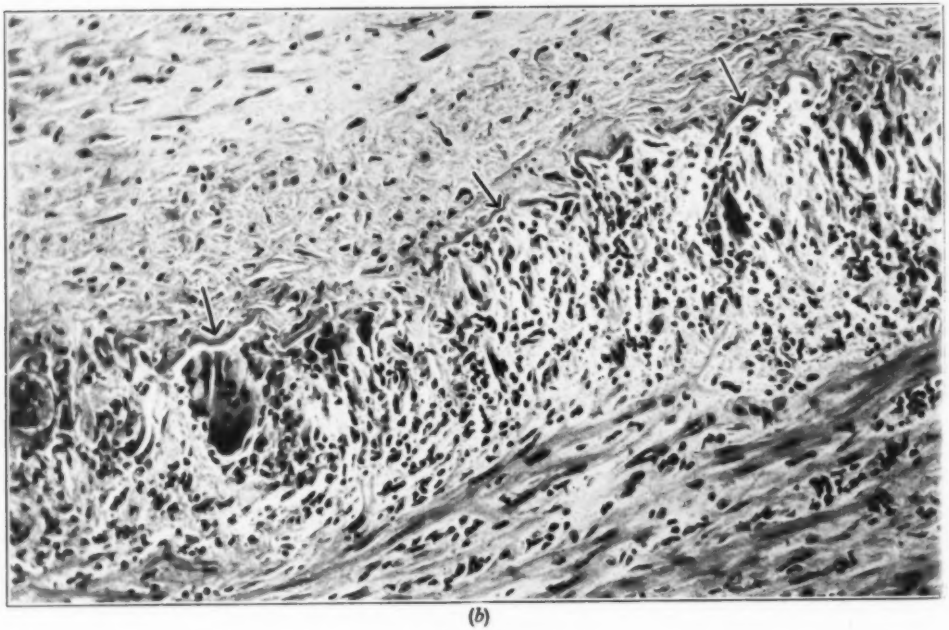

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PLATE II.

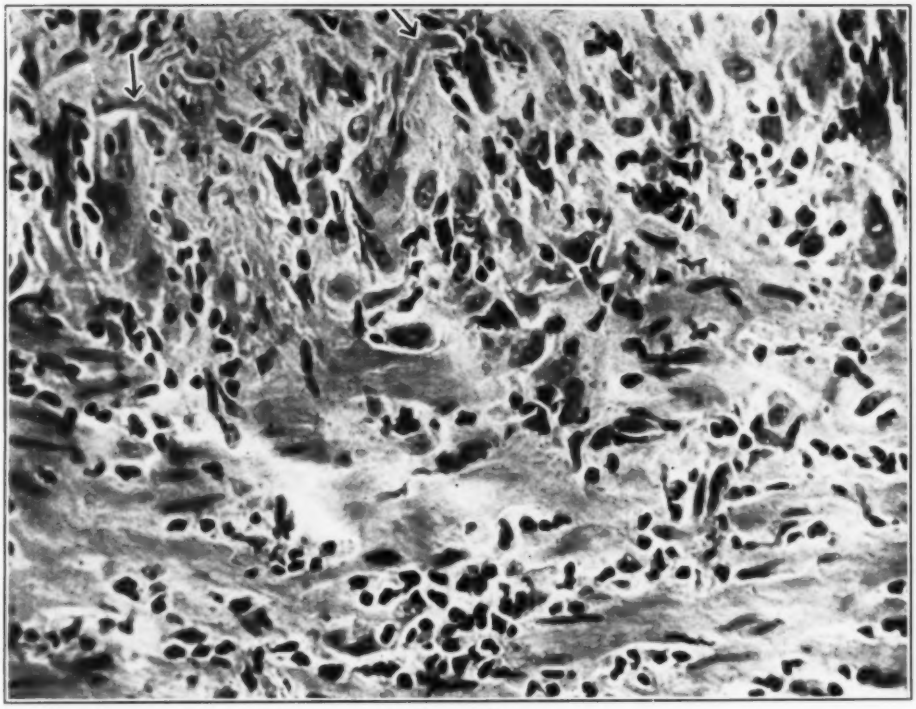
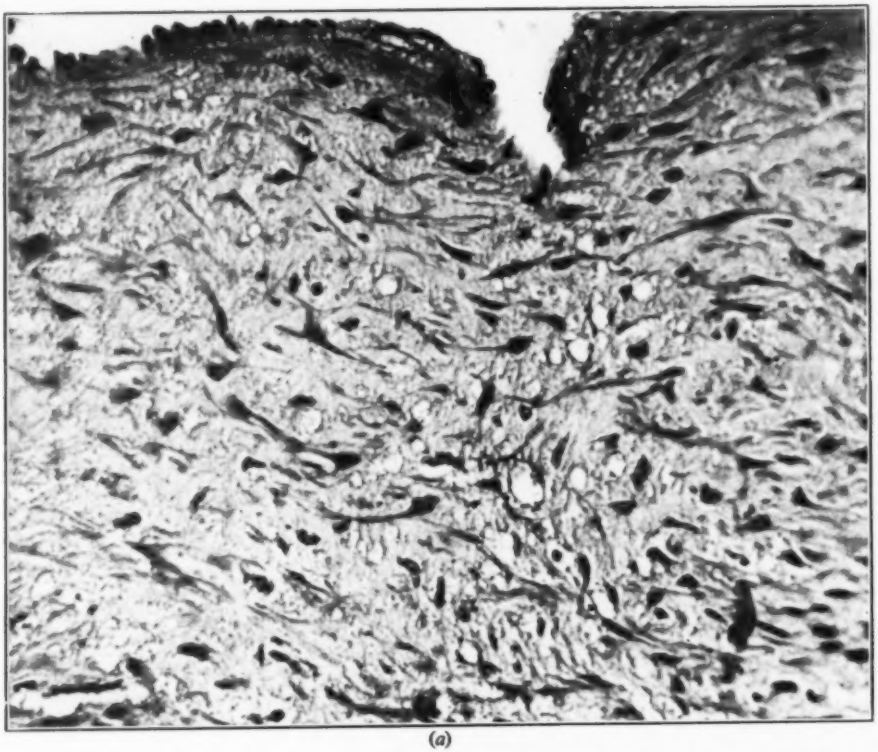


PLATE III.

PLATE I.—(*a*). Section of temporal artery. There is a little mural thrombus adhering to the intima which is greatly thickened. The media is still visible but heavily infiltrated, and it contains many giant cells. The adventitia is fibrosed. (Haematoxylin and eosin, × 40.) (*b*). Same case as (*a*). Showing the fragmentation of the internal elastic lamina and the formation of new elastic fibres in the deeper part of the intima. (Weigert's elastic and neutral red, × 80.)

PLATE II.—(*a*). Same case as Plate I. Showing adventitia and outer part of media. The adventitia is fibrosed and shows a little cellular infiltration. Note two small nerves embedded in fibrous tissue. (Haematoxylin and eosin, × 120.) (*b*). Same case as Plate I. The intima (upper left) consists of cellular fibrous tissue. The internal elastic lamina (arrows) is fragmented. The media is heavily infiltrated with inflammatory cells and there are numerous giant cells near the elastic lamina. Note that the innermost layer of the media is more severely affected than the rest. (Haematoxylin and eosin, × 190.)

PLATE III.—Same case as Plates I and II. Innermost layer of media showing infiltration with lymphocytes and macrophages and the formation of giant cells, apparently from macrophages. The arrows indicate fragments of the internal elastic lamina. (Haematoxylin and eosin, × 430.)



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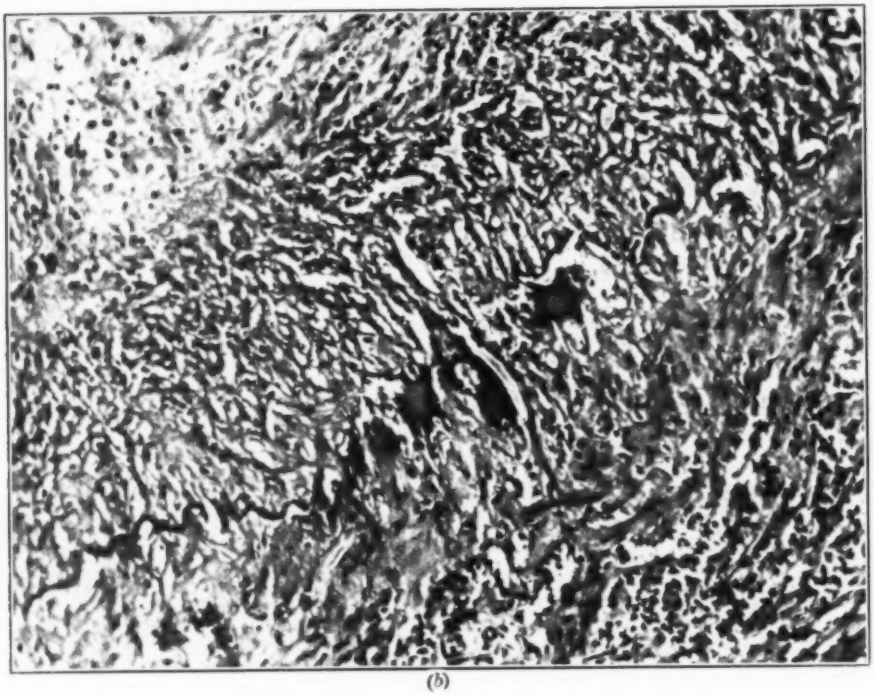

(*b*)

PLATE IV.

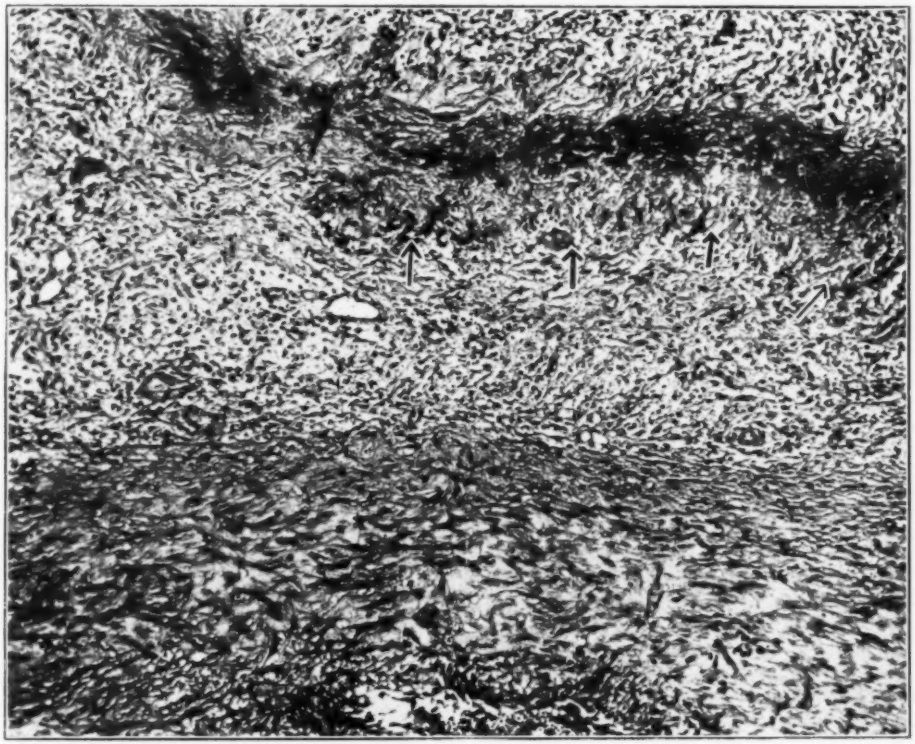

PLATE V.

PLATE IV.—(*a*). Temporal artery. Second case. Innermost layer of thickened intima showing proliferating fibroblasts lying in a background of mucoid connective tissue. (Haematoxylin and eosin, × 460.) (*b*). Same case as (*a*). Junction of intima and media showing giant cells lying against fragments of the internal elastic lamina on its medial side. Note the cellular infiltration of both media and intima. (Verhoeff and Van Gieson, × 190.)

PLATE V.—Temporal artery. Third case. The lumen is just visible at the bottom of the section. The inner part of the intima consists of active mucoid fibroblastic connective tissue. The outer part of the intima consists of vascular granulation tissue. The remnants of the internal elastic lamina are shown by arrows. The media shows a band of necrosis. (Haematoxylin and eosin, × 120.)

excised arteries. Horton and others grew a type of actinomyces from one of their early cases but decided that this was a contaminant, and certainly no one has since grown a similar organism. MacDonald and Moser grew a staphylococcus which they regarded as a contaminant. Other workers (Andersen; Lucien and others; Scott and Maxwell) have failed to grow any organisms from excised arteries. All attempts to grow organisms from blood culture have been unsuccessful (Andersen; Chasnoff and Vorzimer; Schaefer and Sanders). Finally, many workers have searched for organisms in stained sections, all without success. Yet in spite of these completely negative results, there is indirect evidence which suggests that the disease may have an infective basis; it certainly seems to have an association with infection. Gilmour's four cases all began with an influenza-like onset, and Schmidt's dated from an attack of influenza. Dick and Freeman's first case followed an attack of pneumonia, and Paviot and others' case followed the extraction of a septic molar. MacDonald and Moser's case had a dental-root abscess, and Brown and Hampson's was associated with dental sepsis. Dick and Freeman's second case and Hoyt and others' case had faucial sepsis. All these may have been coincidental, but the fact that one of Horton and others' cases recovered after the extraction of septic teeth, and that Profant's case showed a clinical flare-up after the extraction of a septic tooth, strongly suggests that the arteritis and the sepsis were related. Paviot and others' case had further interest in that this subject also had active rheumatic carditis at the time, and both this and the arteritis responded dramatically to salicylates—a form of therapy which no one else records having tried except for the use of aspirin as an analgesic. Lastly, both of Sjövall and Windblad's cases developed crippling arthritis, whilst Andersen's was left with "rheumatoid pain" in his lower limb.

Apart from its apparent associations with infection there are features in the disease itself which suggest infection. The fever, the leucocytosis, the enlarged cervical glands, and the high sedimentation rate, together with the general illness of the patient, all suggest some sort of infective process. Naturally, the failure to find any organism in a disease of an apparently infective type suggests an allergic response, but Dantes (1946) rejects this hypothesis on account of the lack of eosinophils in the lesions and of other signs of allergy in the patients. So far apparently no attempt has been made to isolate a virus. Thus, at present the data available suggest that giant-cell arteritis is possibly of infective origin, but there is no clue as to the type of infection or the mode whereby infection produces the changes.

Comparison with Other Diseases

A number of workers (Jennings; Gordon and Thurber; Kilbourne and Wolff; Hoyt and others; Andersen) have compared giant-cell arteritis with other arterial diseases, particularly thrombo-angiitis obliterans and polyarteritis nodosa. Only with the latter does it bear any close comparison, and the question arises whether giant-cell arteritis has sufficient characteristics of its own to justify regarding it as a separate entity and, if so, how closely it is allied to polyarteritis nodosa. If one analyses the clinical picture and the morphology of the arteries, as some workers have done, then there are many points in common and no feature which is absolutely characteristic of either disease. Nevertheless if giant-cell arteritis—either as a clinical entity or as a histological picture—is viewed as a whole, then there is no question of its not being a real recognizable entity.

The clinical differences between giant-cell arteritis and polyarteritis nodosa depend on two basic factors: age, and the arterial territory involved. Polyarteritis is a disease of young adults and affects the small muscular arteries in the viscera: giant-cell arteritis is a disease of the elderly and affects the elastic and larger muscular arteries proximal to the point where they break up to enter the viscera. The histological differences depend largely on the acuteness of the process. Polyarteritis is often acute, whilst giant-cell arteritis is subacute. The signs of an active infection—the fever, illness, leucocytosis, anaemia, etc.—are common to both. The clinical pictures depend for their differentiation on the territory involved. The differentiating signs of giant-cell arteritis—the headache, palpable temporal arteries, eye involvement, and nervous signs—depend on the involvement of the larger cranial vessels. Similarly the signs of polyarteritis—the renal lesions, cardiac signs, abdominal signs—are the result of visceral ischaemia from involvement of small visceral arteries.

In a similar way the histology of giant-cell arteritis (the lymphocytes and plasma cells, the giant-cells, the fibroblastic reaction) are all those of a subacute disease, whilst the histology of polyarteritis nodosa (the polymorphonuclears and eosinophils, the fibrinoid necrosis, the aneurysms) are all those of an acute process.

There is one final point of difference: polyarteritis is probably an allergic response (Rich and Gregory, 1943; McKeown, 1947); whilst there is no

vidence, either clinical, morphological, or experimental, to suggest that giant-cell arteritis is of this haracter.

Summary

1. Giant-cell arteritis is a disease of the elderly of either sex.

2. It runs a course of about six months and is characterized by malaise, fever, severe headaches nd prominent, thick, painful temporal arteries; t is usually accompanied by leucocytosis, anaemia, nd raised sedimentation rate.

3. There is no specific treatment, but the disease tends to subside slowly and complete ecovery is the rule.

4. It may be complicated by blindness or erebral disturbances from involvement of appropriate arteries.

5. In some cases the disease has proved fatal, nd it has been shown to involve many arteries of he elastic and large muscular group, from the orta down.

6. In most cases a number of cranial arteries are involved.

7. The arterial lesions are characterized by lymphocyte and plasma-cell infiltration of the media with frequent giant cells. The elastica is usually broken up, and the intima undergoes fibroblastic proliferation. There is usually some fibrosis and cellular infiltration of the adventitia. The disease appears to be infective but its aetiology is unknown.

8. The relationship of the disease to polyarteritis nodosa is discussed.

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TEMPORAL ARTERITIS: A REPORT OF THREE CASES

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This disease, which has also been described as cranial arteritis and giant-cell arteritis, was first established as a clinical and pathological entity by Horton and others (1934), though there is evidence that single cases were described before this (Andersen, 1947). Since 1934, over seventy cases have been reported in the literature from U.S.A., Great Britain, France, and Scandinavia. These have been reviewed and analysed elsewhere in this issue (Harrison, 1948). The disease runs a characteristic clinical course, and the affected vessels have a characteristic morphology. Although the earlier recorded cases ran a completely benign course, those subsequently reported have shown that involvement of other vessels may cause blindness, cerebral damage, and even death.

The three cases reported here exemplify the characteristic clinical course and morphology of the disease, and also illustrate some of its complications.

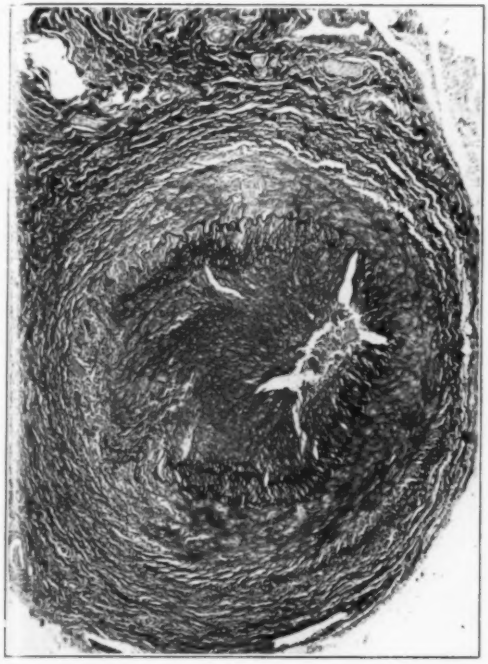
Case Reports

Case 1.—A coach builder, aged 72, from a country district of North Wales was admitted to hospital with a three months' history of shooting pains in both sides of his head. The pain was at first intermittent and located behind the left ear, but later it became almost continuous and spread to the front and back of the head. Six weeks before admission the pain moved to the right temple and came in bursts of great severity alternating with a continuous dull ache. Bending down made the pain worse but coughing and eating did not. During the two weeks before admission the pains had eased a little. On examination he was a well-nourished man with normal temperature and pulse; his face showed leucoderma. Both superficial temporal arteries were visibly prominent but no longer tender. There was no pulsation in either. Physical examination (blood pressure 148/80 mm. Hg.), blood count, electrocardiogram, and radiograph of the chest all gave negative results.

A segment of the right superficial temporal artery was excised, and this appeared to relieve the pain. After nineteen days in hospital, that is, about 15 weeks after the onset of symptoms, the patient was discharged much improved.

Histology (Plate VIa and b).—The lumen was reduced to a small slit by intimal proliferation, but there was no thrombosis. The intimal thickening was composed of a cellular connective tissue containing fibroblast nuclei but very few inflammatory cells. The media was intact around approximately two-thirds of the circumference and was infiltrated by a few inflammatory cells. In the remaining third it was totally replaced by a cellular infiltration of macrophages, lymphocytes, and polymorphonuclears, though there was no remaining necrotic tissue. The internal elastic lamina was fragmented, and there were a few giant cells in the vicinity of the broken elastic fibres. The adventitia was fibrosed, forming a thickened collagenous coat, and several nerves were embedded in it. It did not, however, show any appreciable cellular reaction.

Case 2.—A farmer aged 65 years was admitted complaining of headaches. Six weeks before admission he felt ill and took to bed and thought that he was febrile. Soon after this he developed headaches all over his head but worse on the left side. This was aggravated by coughing and was severe enough to interfere with sleep. He had lost about 1 stone in weight since the onset of his illness. On examination he was found to be well preserved, but he showed signs of recent loss of weight. He had some pyrexia (98 to 101° F.) on admission, but this settled to normal in a few days. The temporal arteries were tortuous, thick, and tender, but pulsating. Blood pressure was 120/80 mm. Hg. Abduction of the right shoulder was limited and the right deltoid wasted. Physical examination was otherwise negative. Blood examination revealed Hb 75 per cent, red blood cells 4,400,000, white cells 12,800 per c.mm. of blood, with 73 per cent polymorphs. The erythrocyte sedimentation rate was 10 mm. in 1 hour (Wintrobe-corrected), the



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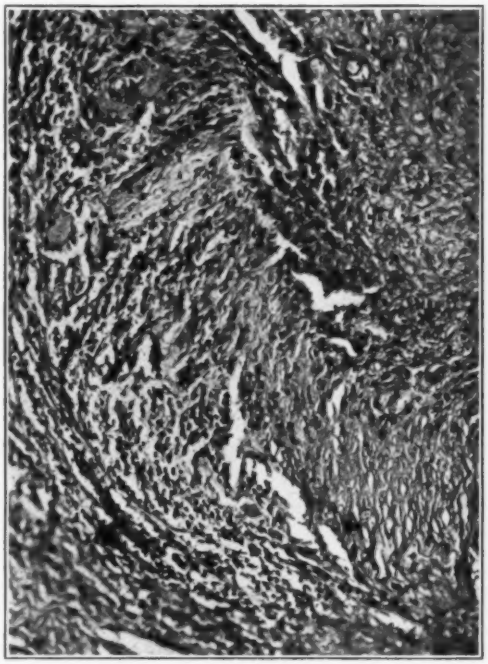

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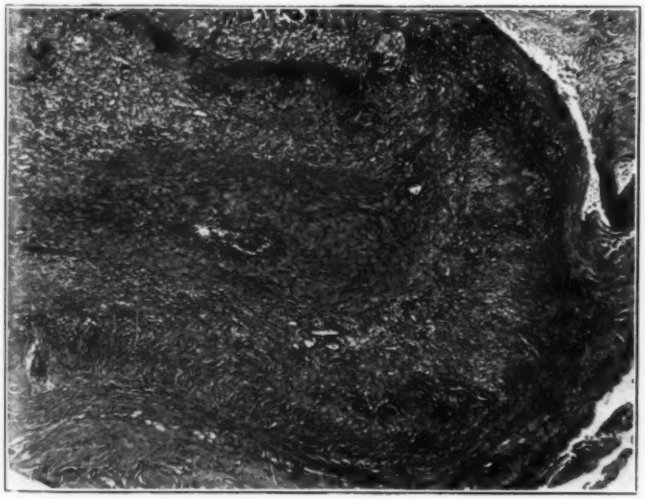

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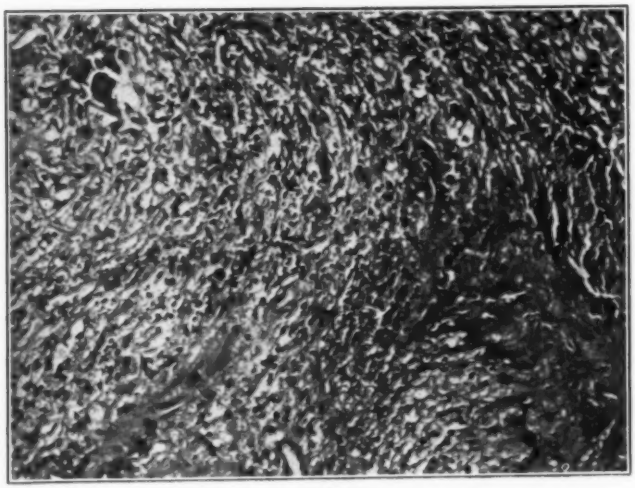
PLATE VI.—(*a*). Case 1. Transverse section of temporal artery showing extreme intimal proliferation. In the segment on the left the media is largely destroyed and the elastica is interrupted. The adventitia is fibrosed. (Verhoeff and Van Gieson, × 50.) (*b*). Case 1. High-power magnification of part of (*a*), showing the cellular infiltration spreading through media. A giant cell is shown near the left side. (Haematoxylin and eosin, × 140.) (*c*). Case 2. Slightly oblique section. The intima is enormously thickened and the inner fibrous and outer granulomatous layers are shown. The media is well seen near the lower border but is necrotic near the upper border. The adventitia is fibrosed, and a small nerve is visible at the extreme lower edge. (Haematoxylin and eosin, × 38.)



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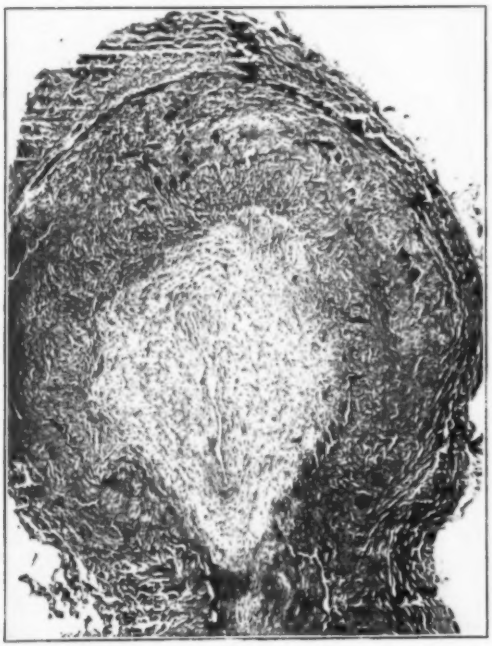

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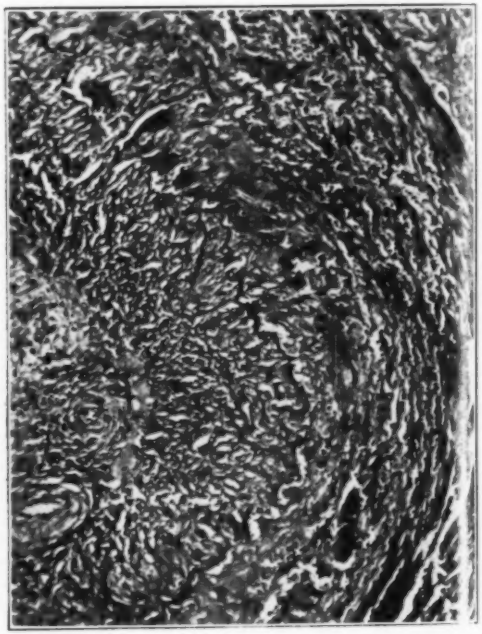

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PLATE VII.—(*a*). Case 2. Higher magnification of part of Plate VIc, showing granulomatous replacement of media spreading into intima (lower left). Two giant cells are seen near the top left corner. (Haematoxylin and eosin, × 190.) (*b*). Case 3. Transverse section just beyond the bifurcation. The greatly thickened intima is composed of loose connective tissue. The media is infiltrated by cells, and the adventitia is fibrosed. (Verhoeff and Van Gieson, × 45.) (*c*). Case 3. Higher magnification of part of (*b*). The intima lies to the left and the media to the right, separated by a wavy black elastic lamina. Note the numerous giant cells in the media. (Verhoeff and Van Gieson, × 120.)

Wassermann reaction negative, and the cerebrospinal fluid normal.

A week after admission a segment of the left temporal artery was excised and this was followed by relief of the headache. A fortnight after admission, eight weeks after the onset of the illness, the patient was discharged free from pain. Six months later he died suddenly from coronary occlusion: no autopsy was performed.

Histology (Plates V, VIc, and VIIa).—The lumen was reduced to a slit 0.1×0.5 mm. but was free from thrombus. The intima was greatly thickened and consisted of two layers. The inner was composed of mucoid connective tissue with fairly numerous fibroblast nuclei but very few inflammatory cells; the outer consisted of granulation tissue with numerous capillaries and fibroblasts and heavily infiltrated with polymorphonuclears and macrophages. The media around approximately half the circumference was necrotic; and the rest, though containing muscle fibres, was heavily infiltrated by polymorphonuclears, lymphocytes, and macrophages. The internal elastic lamina was disrupted, and there was a number of giant cells. The adventitia was fibrosed but contained few inflammatory cells. Several small nerves were embedded in the fibrous tissue. A small branch of the temporal artery included in the biopsy showed exactly the same lesion as the parent vessel. A biopsy of calf muscle did not show any vascular lesion.

Case 3.—A farmer aged 69, and a neighbour of Case 2, was admitted complaining of headache and blindness in one eye. Five months before admission he developed severe pain in the right side of his head; a fortnight later he suddenly developed diplopia for five minutes and then lost the sight in the left eye. A fortnight later the pain on the right cleared up but soon returned on the left side, and he noticed a "swollen vein" in his left temple, which he said was not tender. Two months after the onset of the illness the pain returned to the right side and persisted there till admission. It was severe enough to interfere with sleep.

On examination he was seen to be a wasted elderly man, apyrexial, but with a pulse rate of 100 to 120 per minute. The left superficial temporal artery was thick and tortuous but not tender. The right temporal artery was not palpable. The left eye was blind except for appreciation of light and dark; the disc was pale and there were signs of thrombosis of the central retinal artery. The blood pressure was 175/80 mm. Hg, and there were signs of generalized arteriosclerosis with some myocardial ischaemia. The sedimentation rate was 32 mm. in one hour (Wintrobe-corrected). The blood count showed Hb 90 per cent, red blood cells 4,300,000 and white cells 6,200 per c.mm. The cerebrospinal fluid was normal.

A segment of the left temporal artery was excised and this was followed by relief of his pain. He was discharged free from symptoms a fortnight after admission: five and a half months after the onset of his illness.

Histology (Plates IVa and b, VIIb and c).—The specimen consisted of a Y-shaped segment and was cut to show transverse sections of the two limbs. These were exactly similar. The lumen was patent but reduced to a narrow slit. The intima was greatly thickened and consisted of mucoid connective tissue with frequent fibroblast nuclei but very few inflammatory cells. The media was free from any gross necrosis but was largely destroyed by a granulomatous mass consisting of young capillaries, polymorphonuclears, lymphocytes, and a few macrophages. Giant cells were numerous. This acute inflammatory reaction spread into the immediately adjacent intima and adventitia, but only for very short distances. The internal elastic lamina was broken up into a line of short fragments. Only the inner part of the adventitia was included in the biopsy, but this showed some fibrous thickening. No nerves were included.

Comment

The present cases were all in males, though in the recorded cases there have been slightly more women than men (Andersen, 1947; Cooke and others, 1946). The ages of the present cases, 72, 65, 69, are in accordance with the usual findings, most recorded cases being over 60.

It is of interest that our three cases were country dwellers, and two were neighbouring farmers admitted within three months of each other. Horton and others (1934) drew attention to the fact that their early cases were farmers or country dwellers, though in most of the subsequent publications the patients have either been town dwellers or this information has not been given.

The duration of the disease in our cases was 15, 8, and 23 weeks, which is shorter than the average (about six months) of previously recorded cases. But it is within the range of duration of such cases, which has varied from seven or eight weeks (Hoyt and others, 1941; Sproul, 1942) up to two years' (Cooke and others, 1946).

In this series Case 1 recovered completely. Case 3 also recovered but was left with blindness in his left eye. Case 2 died from myocardial infarction, six months after discharge. An autopsy was not performed, and we do not know whether death was due to arteritis affecting the coronary arteries or to coincidental atheroma. Of the published cases, a number have died within some months of discharge, mostly from myocardial ischaemia or cerebral vascular disease. (Chasnoff and Vorzimer, 1944; Curtis, 1946; Kilbourne and Wolff, 1946). Other cases (Cooke and others, 1946) have died during the course of the disease from cerebral ischaemia due to arteritis of the cranial arteries and the diagnosis confirmed at autopsy.

In the first of the three cases here recorded the lesion appeared to be limited to the temporal arteries; in Case 2 the right circumflex artery was involved, and in Case 3 there was blindness in the left eye due to thrombosis of the central retinal artery. In the early reports it was suggested that the disease was a localized one, but Jennings (1938) described blindness as a complication presumably due to involvement of the retinal vessels, and similar cases have since been reported by Dick and Freeman (1940), Scott and Maxwell (1941), Cooke and others (1946), Shannon and Solomon (1945), Robertson (1947), and Curtis (1946). The cases that have come to autopsy have shown involvement of other vessels.

That the disease is not a localized process is suggested by the frequent occurrence of pyrexia and pains in body and limbs, and a degree of systemic illness out of proportion to the physical findings. Case 2 of the present series was pyrexial and was sufficiently ill to take to bed. The outstanding symptom in our three cases, and in all the recorded cases, has been pain in the head. This is of great severity and is usually resistant to analgesics. Many forms of therapy have been tried but none has been constantly successful. Removal of a segment of the affected temporal artery for biopsy has quite frequently been followed by relief of pain. This was noted by Horton and Magath (1937) and has since been confirmed by numerous other workers, and from the literature it appears to be the most constantly successful form of therapy. It was certainly of value in relieving symptoms in the present three cases. It has been suggested that this is due to interruption of the accompanying nerves, and Lucien and others (1939) described such nerves embedded in the fibrosed adventitia. We have also noted nerve fibres embedded in the fibrosed adventitia of our first and second cases.

Nothing is known of the aetiology of the disease and, though many of the clinical features suggest an infective cause, so far all attempts to isolate an organism have been fruitless. Nevertheless the disease presents a uniform pattern which differentiates it from polyarteritis nodosa or Bürger's disease. It has a different anatomical and age incidence and a better prognosis. The histology of the lesions is also different though in some ways less characteristic. Almost any of the individual histological features of temporal arteritis may be found in either polyarteritis or Bürger's disease, yet the whole picture is sufficiently distinctive to be recognizably different (Gilmour, 1941 ; Cooke and others, 1946).

We are indebted to Mr. F. Beckwith for the photomicrographs.

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THE GASTRIC NEUTRAL-RED EXCRETION TEST

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Introduction

The stomach's acid secretion is commonly used as a measure of gastric function, and various standardized tests have been devised. Owing, however, to the wide range of acid secretion in normal people, to the inconstant results obtained by repeating a test on many individuals, to the frequent occurrence of results well within the normal range in patients suffering from gastritis, gastric carcinoma, or peptic ulcer, and to the fact that from 10 to 20 per cent of normal people may have spontaneous temporary or permanent achlorhydria (Schiff, 1938; Palmer and others, 1940), estimation of the acid secretion of the stomach after a popularity of many years is now falling into disfavour as a diagnostic test.

In the search for a more reliable test, the excretory function of the gastric mucosa to various substances including dyes has been studied: one of these dyes is neutral red.

When a solution of neutral red is injected into a vein of a normal individual, the dye is *excreted* within a few minutes into the gastric juice, staining it pink: the concentration increases for a period and thereafter diminishes. During the next twenty-four to forty-eight hours the dye is excreted in the urine and colours the faeces.

In 1923 Glaessner and Wittgenstein introduced the excretion of neutral red by the stomach as a test of gastric function, but the normal variations were not worked out, nor was the relationship of dye *excretion* to acid *secretion* fully understood by these or subsequent workers (Davidson and others, 1925; Zibalis, 1934; Winklestein, 1942; Lourja and Mirkin, 1925). Twenty years later the excretion of neutral red by the stomach was reintroduced by Gillman (1943, 1944) as a test of gastric function. He used a standard technique whereby the rate of dye excretion and the rate and intensity of concentration of the dye in the gastric juice could be measured. He worked out the normal variations in healthy young European adults in Johannesburg, and showed that normal results were repeatable within narrow limits. By studying the results obtained on three hundred cases, mostly African natives with disturbed gastric function, he claimed to have established criteria of abnormality which could be of diagnostic and prognostic aid.

The intention of the present study was to check the range of normality reported by Gillman; to study the anatomy and physiology of neutral-red excretion; to compare the acid secretion of the stomach with its power to excrete dye, and to determine if the test could be of use in this country in the diagnosis of various gastric and duodenal disorders.

Methods

Technique of the neutral-red excretion (N.R.E.) test.—The Gillman method was adhered to with only minor modifications. A Ryle or Rehfuss tube was passed through the mouth into the stomach of the patient, who had fasted overnight. After the resting juice had been drawn off, the stomach was washed out with 100 ml. or more of warm water by means of a 20-ml. syringe until the aspirate became clear. When little or no mucus appeared in the washing, the stomach was emptied and was ready for the test. In a few cases, especially in carcinoma of the stomach, it was found difficult or impossible to obtain a clear and clean aspirate: these were cases with great excess of mucus in the juice, often together with altered blood.

Without delay, 5 ml. of a 1 per cent sterile neutral-red aqueous solution was injected intravenously as rapidly as possible, and a stop-watch was started. Any remaining stomach contents were removed as soon as the injection was completed. Subsequent aspirations were carried out at 1-minute intervals

until the dye first appeared in the juice. After that, aspiration was repeated every 2 minutes for the next 15 to 20 minutes, and later at 3- to 5-minute intervals. The period of the test was at least 30 minutes, and usually ten to fifteen aspirates were made. The entire volume of gastric contents obtainable was removed at each aspirate and placed in a test-tube of 5/8 in. diameter upon which was marked the number of minutes from the time of the dye injection. The complete removal of the gastric contents before and immediately after injection and at subsequent aspirations was considered important; otherwise an unknown dilution factor would occur. Patients were instructed to expectorate and not to swallow saliva excreted during the test.

Difficulty in aspiration sometimes occurred: if the tube was blocked by a plug of mucus, injection of 20 ml. or more of air down the tube often cleared it: sometimes aspiration was facilitated by the patient bending forward.

The concentration of the dye in each sample of gastric juice was estimated by direct vision in a comparator box using standard dilutions of the dye contained in identical test-tubes. The standard tube was backed with a tube containing uncoloured juice, and the unknown with a tube of water. With a little practice the dilutions were easy to read.

The standards were kept in well-stoppered test-tubes ready for use. Deterioration in colour was not appreciable for three or four months. The dye was made up in decinormal hydrochloric acid and the following dilutions used, viz., 1/300,000, 1/200,000, 1/100,000, 1/75,000, 1/50,000, 1/30,000, and 1/20,000. The actual concentration of the dye was not usually recorded but the arbitrary scale of Trace, 1, 2, 3, 4, 5, and 6 used by Gillman was adopted (Trace = 1/300,000, 1 = 1/200,000, etc.). Graphic representation of each result was made by plotting dye concentration of the sample against the time in minutes (see Figure).

Further studies of the fasting juice and subsequent unstained and stained gastric fractions were carried out. The volumes were measured, the presence of mucus, bile, and macroscopic blood were noted, and benzidine tests for occult blood were performed. "Free hydrochloric acid" and "total acid" estimations were performed on the fasting specimen and on four or five samples of juice removed at consecutive 5- to 10-minute intervals. The dye was first removed from the sample by filtration through two or three thicknesses of filter paper, which absorbed the neutral red. Sometimes refiltration was necessary. Titration was carried out on 5 ml. (or if available 10 ml.) of the specimen with N/10 or N/20 sodium hydroxide, using a drop or two of a mixture of Töpfer's reagent and phenolphthalein as indicators. If achlorhydria was present, qualitative tests for pepsin were performed on two or three mixed samples of juice using coagulated egg-white as a substrate.

In some of the cases (*vide infra*) in which excretion of neutral red did not occur or was very poor within the first 30 minutes, aspirations were continued every 5 minutes for a further 30 to 60 minutes, sometimes after an intramuscular injection of 0.5 mg. of histamine.

Fractional test meals.—These were not performed on the twenty "control" cases, but were carried out on patients exhibiting gastric symptoms, usually a few days before the neutral-red excretion test was performed.

Absence of toxicity.—In the dosage used, neutral red was found to be free from toxic or unpleasant local or general effects. Its excretion in the urine and its colouring of the faeces within the following day or two was physiological. It was found advisable, however, to warn the nursing staff and the patient of this occurrence so as to prevent undue anxiety or alarm.

Observations

Results in N.R.E. tests in patients free from gastro-intestinal symptoms.—N.R.E. tests were performed on twenty in-patients of a military hospital who were free of any gastro-intestinal symptoms or signs. It was difficult to find perfectly healthy patients in hospital, but patient volunteers with various non-gastric disorders were selected. They consisted of six convalescent minor surgical

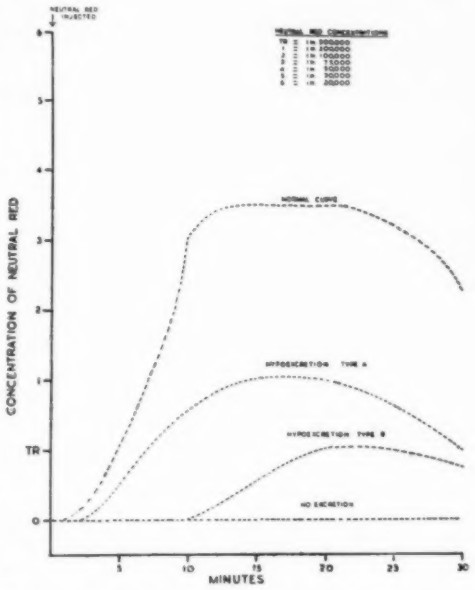

FIGURE.—Normal and abnormal neutral-red excretion curves.

PLATE VIII.—Photograph of a colour drawing showing excretion of neutral red by the mucosa of the upper half of the body of the stomach (black streaks = neutral red).

(*Original drawing by Miss D. Davidson.*)

and fracture cases, three patients completely recovered from old head injuries, two mildly neurotic patients, and a case each of Bell's palsy, tinea pedis, convalescent atypical pneumonia, endometrosis, convalescent tonsillitis, progressive spastic palsy, congenital cystic disease of the lungs, and convalescent catarrhal jaundice. All these patients were out of bed; they were fit and well and had good appetites.

Only two of the patients were women; of the men, four were Germans, one an Italian, another a Pole, and the remainder were English, Scottish, or Irish. Two patients were 35 and 48 years of age respectively, the remainder 18 to 26 years old.

The details and shape of the N.R.E. curves were studied (see figure). The general shape of each curve was similar; dye tinged the juice a faint pink within a few minutes, and the concentration increased—usually quite rapidly—to reach a maximum which was maintained for a period and which then declined. Particular attention was paid to:

(1) The *excretion time* (E.T.), or the period in minutes between injection of the dye and its first appearance in the aspirate. In all cases this was 5 minutes or less. It is noteworthy that in seven cases dye appeared within 1 minute, in thirteen within 2 minutes, and in seventeen cases within 3 minutes of the injection.

(2) The *concentration time* (C.T.) or the period in minutes between injection of the dye and its earliest maximum concentration in the juice. In fifteen cases this was between 8 and 12 minutes; in the remainder it was 4, 5, 6, 14, and 19 minutes respectively.

(3) *The maximum degree of concentration found.* (C_M). Using the arbitrary scale of Trace, 1, 2, — 6 (see above), the C_M in 15 cases was between 3 and 5; one was 6, three were 2, and one was 1.

TABLE I

RELATIONSHIP OF NEUTRAL-RED EXCRETION TESTS TO THE ACID SECRETION OF THE STOMACH

Group of cases	No. of cases in group	Classification of N.R.E. tests	No. with hyper-acidity	No. with normal acidity	No. with hypo-acidity	No. with achlor-hydria	Total
Non gastric controls	20	Normal N.R.E.	5	14	0	0	19
		Hypo-excretory, Type a	0	1	0	0	1
Carcinoma of stomach	10	Normal N.R.E.	1	2	0	0	3
		Hypo-excretory, Type b	0	0	0	2	2
		N.R. not excreted	0	0	1	4	5
Chronic dyspepsia and chronic gastritis	11	Normal N.R.E.	0	4	1	0	5
		Hypo-excretory, Type b	1	1	0	0	2
		N.R. not excreted	0	1	0	3	4
Peptic ulcer	16	Normal N.R.E.	4	6	0	0	10
		Hypo-excretory, Types a and b	0	4	2	0	6
		N R. not excreted	0	0	0	0	0
All Cases	73	Normal N.R.E.	11	35	2	0	48
		Hypo-excretory	1	6	2	3	12
		N.R. not excreted	0	1	2	10	13
Totals			12	42	6	13	73

Statistical Analysis—Chi-squared testing of all cases was done by grouping of classes owing to the small numbers. In all arrangements used there is a highly significant departure from random distribution (χ^2 = 35.06, 31.3 or 30.09: $P < 0.0001$). The chief contribution to the Chi-squared comes from the achlorhydric cases.

Case No.
9
21
58
62
69
71
72
59
2
4
10
28
66
69
51
68
8
25
42
20
50
56
6
61
14

TABLE II

ANALYSIS OF CASES WITH ABNORMAL N.R.E. TESTS

Case No.	Diagnosis	*X*-ray findings barium meal, etc.	Laparotomy findings and operation	N.R.E. test			Max. free HCl of gastric juice
				E.T.	C.T.	C_M.	
9	Non-gastric control (Bells palsy)	—	—	1	9	1	62%, Normal acidity
21	Gastric carcinoma	Filling defect near lesser curvature	—	Never	Never	Nil	Achylia gastrica "coffee-ground" fasting juice
58	Gastric carcinoma	Irregular filling defect on lesser curvature	Inoperable cancer of lesser curvature	Never	Never	Nil	Achlorhydria
62	Gastric carcinoma	Large ulcer on lesser curvature	Malignant ulcer; secondaries in liver	Never	Never	Nil	8% hypo-acidity
69	Gastric carcinoma	Ulcer on lesser curvature	High, inoperable carcinoma	Trace 9 and 11 mins.	Never , Intermittent excretion	1 at 13 mins.	Achlorhydria
71	Gastric carcinoma	Large, pyloric filling defect	Pyloric cancer: partial gastectomy	Never	Never	Nil	Achlorhydria
72	Gastric carcinoma	Filling defect near cardia	Inoperable carcinoma	Never	Never	Nil	Achlorhydria
59	Carcinoma of transverse colon	Filling defect on greater curvature	Malignant infiltration of greater curvature	20 Intermittent excretion	Never	1	Achlorhydria
2	Chronic dyspepsia	Normal	—	24	Never	Trace	50%, climbing curve in F.T.M. Mucus + + +
4	Alcoholic gastritis (with hepatic cirrhosis)	Normal	—	Never	Never	Nil	Achylia gastrica
10	Chronic gastritis	Normal	—	Never	Never	Nil	Achylia gastrica
28	Chronic gastritis	Normal	—	10 Intermittent excretion	Never	Trace	70% climbing curve in F.T.M. hyperacidity
66	Chronic dyspepsia	Mucosal puckering onlesser curvature, ? gastric ulcer	Stomach, duodenum etc., normal	Never	Never	Nil	42% normal acidity
69	Alcoholic gastritis (with renal colic)	Normal	—	Never	Never	Nil	Achlorhydria
51	Chronic cholecystitis	Normal	—	Never	Never	Nil	Achlorhydria
68	Chronic cholecystitis	Barium meal = normal. Gastroscopy = atrophic gastritis	Cholecystectomy and appendicectomy	Never	Never	Nil	Achlorhydria
8	Gastric ulcer	Ulcer on lesser curvature	—	9	Never	Trace	Trace of free HCl, Pepsin ±. "coffee-ground" fasting juice
25	Gastric ulcer	Ditto	—	3	7	1–2	12% low normal acidity
42	Gastric ulcer	Ditto	—	9	35	1	9% hypo-acidity
20	Duodenal ulcer	Shallow ulcer on medial border of cap	—	2	Never	Trace	62% normal acidity
50	Duodenal ulcer	Ulcer on cap	Partial gastrectomy	5	28	2	60% normal acidity
56	Gastro-jejunal ulcer	(Gastro-enterostomy several years previously)	Vagotomy	Trace 11–20 mins.	25	2	15% low normal acidity
6	Intestinal colic	Barium meal and enema = N.A.D.	—	31	Never	Trace	Achlorhydria Pepsin +
61	Achalasia cardia	Barium meal indefinite	—	Never	Never	Nil	10% hypo-acidity
14	Post-head-injury depression	Normal	—	Never	Never	Nil	Achlorhydria, Pepsin ±

E.T. = Excretion Time. C.T. = Concentration Time. C_M. = Maximum Concentration.

The normal curve of N.R. excretion.—Excluding one test, excretion of neutral red by the stomach began within 5 minutes of the injection; the dye was then concentrated to reach a maximum within 19 minutes, and the maximum concentration reached was between 2 and 6. In the case of Bell's palsy showing a C_M of 1 only, the E.T. was 1 minutes, C.T. 9 minutes (see Table II).

Except for the maximum degree of concentration, these results are in general agreement with those reported by Gillman (1944). He found that normal individuals excreted dye into the stomach within 10 minutes of the injection (usually 4 minutes) and concentrated it to an intensity of 4 to 6 within 20 minutes (average 14 minutes). If Gillman's maximum concentration is to be accepted, then the ten N.R.E. curves with a maximum of 3, 2, or 1 would be considered abnormal. None of these patients had any symptoms or signs of gastro-intestinal disorder, and they formed 50 per cent of the present "control" series.

It was arbitrarily decided to consider a C_M of 1 as abnormally low and to define a normal N.R.E. curve as one in which dye is excreted within 10 minutes and reaches a maximum concentration of 2 to 6 within 20 minutes of intravenous injection of 5 ml. of 1 per cent neutral red.

Neutral-red excretion tests in patients with gastric or intestinal disorders.—Tests were performed in a military hospital and in the Professorial Surgical Unit, Manchester Royal Infirmary, on fifty-three patients with disturbed gastric or intestinal function. The cases fell into eight groups, viz.: gastric carcinoma, peptic ulcer, "duodenitis," gastric neurosis, lienteric diarrhoea, chronic dyspepsia including chronic gastritis and alchoholism, cholecystitis, and a miscellaneous group.

The ten patients with carcinoma of the stomach were all in the 40 to 70 years age group; six were men. In eight patients clinical and *x*-ray diagnosis was confirmed by laparotomy. One patient had primary carcinoma of the transverse colon with direct malignant infiltration of the greater curvature.

Of the sixteen patients with peptic ulcer, fourteen were men: six were under 30, seven between 30 and 50, and three were more than 50 years of age. Diagnosis of duodenal, gastric, or gastrojejunal ulcer was made by correlating clinical symptoms and signs with *x*-ray evidence following a barium meal. In ten cases laparotomy was performed and the diagnosis confirmed.

The three patients with "duodenitis" were all men under 30 years old. Diagnosis was made when classical symptoms of duodenal ulcer of recent origin were present, and when no *x*-ray evidence of peptic ulcer was found.

Of the four cases diagnosed as gastric neurosis, three were men: all were under 25 years of age. In three an anxiety state was present: the other was a case of depression with vague gastric symptoms following a head injury.

The three cases with lienteric diarrhoea were all young men: each gave a classical history of lienteric and post-pandrial diarrhoea of several years' duration. Clinical and *x*-ray examinations were negative.

The chronic dyspepsia-gastritis group was a heterogenous group of ten men and one woman. Four were under 30 years of age, three between 30 and 50, and the remainder older men. Three of the patients were chronic alcoholics and had morning anorexia with flatulent dyspepsia (one also had mild polyneuritis and liver cirrhosis); the remainder gave a history of dyspepsia (flatulence and/or discomfort after meals) with or without anorexia of some years' duration. In some cases the history was typical of chronic gastritis, but in others a satisfactory diagnosis could not be made. Cardiovascular disease was minimal or absent, and neither clinical nor *x*-ray examination was of positive help. In three cases, laparotomy revealed no abnormality.

The diagnosis of cholecystitis in one case (male, age 59 years) was made on clinical grounds and a negative barium meal radiograph: in the other (a woman, age 62 years), the barium meal radiograph showed diminished motility of the stomach, and gastroscopy an atrophic gastritis. At laparotomy, cholelithiasis was found and cholecystectomy and appendicectomy were performed.

The miscellaneous group were cases of subacute nephritis, repeated attacks of acute gastritis, achalasia of the cardia, and severe intestinal colic of unknown origin.

Results of N.R.E. tests.—Tests on twenty-nine of the fifty-three patients were within the normal range. The abnormal results fell into two groups, viz.: those in which there was no excretion of dye into the stomach (anexcretory type) (13 cases), and those in which the dye was poorly concentrated (11 cases). This group could be subdivided into two partially overlapping subgroups: (a) those with a normal E.T., C_M less than 2 and a normal or delayed C.T., and (b) tests with normal or delayed E.T. and C_M 1 or less. The dye excretion in three was slight and intermittent. For descriptive purposes, these will be called hypo-excretory types a and b. The figure portrays examples of

these curves in contrast with a normal result, and one in which the dye was not excreted.

Table I classified the tests according to diagnosis, the non-gastric controls being included for comparison. Table II is a more detailed case and test analysis of the patients with abnormal N.R.E. tests.

Of ten patients with gastric carcinoma, five failed to excrete neutral red ; in two excretion was poor and intermittent. Four of the eleven patients with chronic dyspepsia or gastritis did not excrete the dye, in two excretion was poor and delayed or intermittent. Both cases of cholecystitis were of the anexcretory type, but one patient also had atrophic gastritis. Of the sixteen peptic ulcer patients, ten had normal N.R.E. tests, the remainder hypoexcretory curves. Each case of "duodenitis" and lienteric diarrhoea, and three of the patients with gastric neurosis excreted neutral red normally. One of the latter (post head-injury depression) failed to excrete the dye. Of the four miscellaneous cases, the patient with achalasia of the cardia did not excrete neutral red and the patient with undiagnosed intestinal colic had only a very delayed and slight excretion of dye.

From these results it is clear that abnormal N.R.E. tests of the anexcretory and hypoexcretory types may occur in various organic disorders of the stomach and duodenum. Separate types of N.R.E. curves were not detected for particular diseases, and therefore the test cannot be of help in discriminating between (say) gastric carcinoma and gastritis. It is doubtful whether the test can distinguish between organic and functional disorder of the stomach, as cases of achalasia of the cardia (a local nervous disorder) and post-head-injury depression (with functional gastric symptoms) had anexcretory results. Moreover, normal N.R.E. tests can be given by patients with well-established organic disease of the stomach such as gastric carcinoma or peptic ulcer.

In practice the N.R.E. test is of value when an abnormal result is obtained: the conclusion may then be drawn that there is a disorder of the gastric mucosa of organic, functional, or possibly reflex origin.

Gillman (1944) found that normal results were repeatable after variable intervals of time. To confirm this, nine patients were subjected to a second test two to five weeks after the first. Seven had given normal N.R.E. curves on the first occasion and when repeated these tests were still within normal limits ; for example Case 3 (chronic gastritis) excreted neutral red at 3 minutes and concentrated it to a maximum of 2–3 in 14 minutes: five weeks later, excretion of dye began in 2 minutes and reached a maximum of 3 in 8 minutes. Two other patients failed to excrete the dye both at the first tests and when these were repeated a few weeks later.

Anatomy and physiology of neutral-red excretion.—Attempts were made to compare the blood level of the dye with its concentration in the gastric aspirate. In a patient with a blood volume of (say) 7 litres, the injection of 5 ml. of 1 per cent solution should produce a blood level of dye of about 1 in 140,000 or a plasma level of about 1 in 270,000 if all the dye remains in the plasma and if none diffuses into the extracellular tissues or is lost by excretion. *In vitro* experiments showed that when neutral red is added to oxalated whole blood, almost the entire quantity is present in the plasma, where its concentration could be estimated colorimetrically up to a concentration of 1 in 250,000 and detected up to 1 part per million.

Venous blood was removed from eight patients (from the arm opposite to the injection) 5 to 20 minutes after injection of the dye. In no case was it possible to detect neutral red in the plasma, although at the same time dye was being excreted in the juice in concentrations of 1/50,000 to 1/200,000. In two patients from whom blood was removed 5 and 6 minutes after injection, the dye was just appearing in trace concentration in the gastric juice. The urine was free from dye and it was considered impossible that excretion of dye could have been responsible for a plasma level of below 1 part per million. It was therefore concluded that neutral red rapidly diffuses into the extracellular tissues of the body, is present in a concentration of less than 1 in a million in the plasma, and is concentrated by the gastric mucosa during excretion at least 10 to 20 times.

In a patient with a duodenal ulcer (Case 49), the site of neutral-red excretion was established by a method suggested to one of us by Professor A. M. Boyd. This patient had an ulcer history of twenty years' duration, verified by barium meal *x*-ray examination. The fractional test meal showed hyperacidity and the N.R.E. test performed the day before the operation was normal (E.T. 2 minutes, C.T. 4 minutes, C_M 4).

Premedication with omnopon, 1/3 gr., and scopolamine, 1/150 gr., was given an hour and a half before operation which was carried out under ether anaesthesia. Partial gastrectomy was performed: during the course of the operation 5 ml. of 1 per cent neutral red was injected intravenously as soon as the greater curvature had been freed and the right gastric artery ligatured. Within a few minutes, the upper half of the gastric mucous membrane was stained red by the dye (see Plate

VIII) and there was a fairly sharp line of demarcation halfway down the stomach below which no dye was excreted. The anexcretory area included the lower half of the body of the stomach, the pyloric antrum, and the pylorus. Excretion of dye still persisted when the surface was washed with saline.

Was the lack of the excretion in the lower half of the stomach due to tying the right gastric artery and freeing of the greater curvature? To exclude this possibility the injection of dye was repeated several times during the course of further gastrectomies for peptic ulceration and the same distribution of dye excretion was obtained notwithstanding when the dye was injected or its relationship to the tying of vessels.

Was the lack of excretion in the lower half due to antral gastritis? The gastric mucosa looked normal and subsequent histological examination of various areas including the antrum showed no abnormality.

Evidence of the nervous control of dye excretion was obtained in other cases. In a patient with a gastro-jejunal ulcer (see Table II, Case 56) there was a low normal gastric acidity before operation, and excretion of neutral red occurred although this was delayed and rather poor. Four weeks after a succcessful transpleural vagotomy the tests were repeated. No excretion of dye was found, and the patient had achlorhydria.

N.R.E. tests with normal results were performed on five patients, who for various reasons were later submitted to sympathectomy: three patients had unilateral and two bilateral sympathectomy from T 5 to L 3 performed; splanchnicectomy was also carried out. The tests were repeated 2 to 3 weeks later and no significant changes in the excretion or concentration of dye by the stomach was found.

Several unsuccessful attempts were made both in rabbits and in man to discover the cellular origin of dye excretion. Biopsies of the stomach wall from the fundus, body and pylorus were taken within a few minutes of an intravenous injection of neutral red. The human material was collected during the course of partial gastrectomies. Frozen and paraffin sections were made and examined, stained and unstained, but no intracellular dye was seen even after treatment of the section with acid (neutral red is yellow in alkaline solution).

These results indicate that the excretion of neutral red is limited to the upper half of the gastric mucosa, that excretory activity is abolished by vagotomy, and that the sympathetic plays no part in the control of the excretion. The nervous control of the dye excretion and acid secretion is identical. Hydrochloric acid is secreted by the mucosa of the body of the stomach, probably over a wider area than that responsible for neutral-red excretion. The parietal (oxyntic) cells are not present in the pyloric region where an alkaline fluid is secreted. Stimulation of the vagus nerve in dogs produces a flow of strongly acid juice, whilst stimulation of the sympathetic splanchnic nerves gives only a slow steady secretion of fluid rich in mucus, often alkaline in relation (Pavlov, 1910; Carlson, 1923; Babkin, 1938). This latter secretion comes mainly from the pyloric glands and only to a minor extent from the body of the stomach.

From similar anatomical sites of acid secretion and neutral-red excretion, and from their identical nervous control, it might be expected on theoretical grounds that a relationship between acid secretion and dye excretion exists, and that an abnormality in one property would be accompanied by a parallel abnormality in the other, for example achlorhydria with failure to excrete the dye.

Relationship of acid secretion to neutral-red excretion by the gastric mucosa.—"Free" and "total" acid estimations were performed on specimens of gastric juice from all patients, including the controls. Forty of the cases with disturbed gastric or intestinal function had fractional test meals performed: in fifty cases acidity was estimated on several specimens of gastric juice obtained during the course of the N.R.E. test, and in four of these acid estimations were performed on juice obtained after an injection of histamine.

Acid secretion of the stomach has a wide range of normality, and it is therefore difficult to decide when abnormality begins. Arbitrary figures have to be adopted, and it was decided to accept a free acid equivalent to 70 ml. of N/10 hydrochloric acid per cent (70 per cent) as the limit of the upper range. Similarly the limit of the lower range was accepted as a free acid volume of 10 ml. of N/10 hydrochloric acid per cent (10 per cent). If one or more fractions of juice had a value of 70 per cent or over, *hyperacidity* was said to be present: if *all* the specimens of juice analysed were less than 10 per cent, *hypo-acidity* was said to exist.

From an examination of Tables I and II it is clear that the ability of the stomach to excrete neutral red and to secrete hydrochloric acid are not *necessarily* allied. Six patients (Cases 2, 9, 20, 25, 50, and 56) with normal acid production had hypo-excretory dye curves, one patient with hypo-acidity excreted the dye poorly (Case 28), and another (Case 66) with normal acid production

failed to excrete the dye at all. Gillman's (1944) statement that patients with very poor or no excretion of dye are always achlorhydric is incorrect. However, there is a statistical correlation between normal acid production and normal neutral-red excretion and between achlorhydria and lack of excretion of dye. This relationship is probably the reflection of a common anatomical mucosal site for secretion and excretion and of a common nervous control of these activities.

Conclusions and Summary

The excretion of neutral red by the normal gastric mucous membrane follows the same pattern in different people within fairly narrow and easily recognized limits. Normality and abnormality in dye excretion is readily established using the neutral-red excretion test standardized by Gillman. When the test is repeated on the same individual, identical or closely similar results are obtained whether dye excretion is normal or abnormal. Rarely is an abnormal N.R.E. test given by a patient with a healthy gastric mucosa, and it can be said that abnormality in excretion of the dye is strongly suggestive of disordered gastric function.

For these reasons the N.R.E. test is superior and more reliable than the estimation of acid secretion. Other material advantages include safety, simplicity, and the short time it occupies. Furthermore, the dye and the apparatus are easily obtained, and if necessary the test can be performed by the clinician at the bedside.

All investigators agree that total failure to excrete neutral red is diagnostic of gastric dysfunction and many (Lourja and Mirkin, 1925; Morrison, 1938; Gillman, 1944) that partial suppression of dye excretion is an abnormal result preceding the final stage of total suppression. Whilst the present investigation is in the main in agreement with these observations, there is no doubt that patients with various disorders of the stomach and duodenum, including carcinoma and peptic ulcer, may have normal dye excretion. This may occur notwithstanding the duration of the disorder or the severity of the condition. A normal result is then of no value in distinguishing a normal from a pathological stomach, and the test is not nearly as sensitive as Gillman suggests.

Furthermore, neither a normal nor an abnormal result is of diagnostic use in differentiating between cancer, gastritis, and other conditions. Although the results of the peptic ulcer series suggest that complete suppression of excretion does not occur in cases of simple ulcer, this may not be true and may result from pure chance in the selection of the cases. Functional disorder of reflex or nervous origin may or may not suppress excretory function, so that the N.R.E. test is of no value in distinguishing organic from non-organic disease.

The problem of serial tests on the same patient to determine the deterioration or recovery from gastric disorder was not studied.

Although a statistical relationship exists between excretion of neutral red and secretion of acid, the correlation has exceptions and in any individual case disorder of one function may or may not be accompanied by a parallel disorder of the other.

Finally, experiments and observation indicate that, following the injection of neutral red, most of the dye rapidly diffuses into the extracellular tissue fluid and is present in the blood in minute undetectable traces (concentration < 1 part per million) although at the same time it is being excreted by the gastric mucosa in concentrations up to 1 in 50,000. The parasympathetic vagus nerves are probably the excitory nerves controlling dye excretion, and the sympathetic plays no part in the activity.

The investigations of one of us (S.S.) were carried out at the Military Hospital for Head Injuries, Wheatley, Oxford, and of the other (R.P.J.) in the Professorial Surgical Unit, Manchester Royal Infirmary. We are indebted to many clinical and laboratory colleagues and to members of the nursing staff of both hospitals for their co-operation and assistance. In particular we wish to thank Dr. J. Bull for his statistical assistance in the analysis of part of Table II, Prof. A. M. Boyd for his timely suggestions and co-operation during the pursuance of the work in Manchester, and Miss D. Davidson for her excellent drawing. We are indebted to Lieut.-Col. E. H. Hall, R.A.M.C., A.D.P. Southern Command, for his interest. We acknowledge the permission of the Army Medical Department, War Office, to publish this investigation.

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THE FEULGEN REACTION APPLIED TO CLINICAL HAEMATOLOGY

BY

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The Feulgen reaction is a cytochemical test for the detection of desoxyribonucleic (thymonucleic) acid. After acid hydrolysis, desoxyribonucleic acid in the presence of sulphurous acid gives a characteristic red-purple colour with basic fuchsin, and the depth of the colour roughly reflects the concentration of the compound. The detailed chemistry of the reaction has been fully described by Baker (1942). The reaction has been known for fifteen years and is much used in biology, especially in genetics, but it has only recently been applied to the problems of clinical haematology: La Cour in 1944 used the reaction to study mitotic abnormalities that occur in the bone marrow cells in pernicious anaemia; Thorell working in Caspersson's laboratory in Stockholm used the Feulgen reaction together with ultra-violet absorption techniques for studying the intimate metabolism of nucleoproteins in blood-forming cells (Thorell, 1944, 1947). We have utilized the Feulgen reaction to give help in classifying blood and bone-marrow cells when the differentiation is of clinical importance and when the routine methods fail to show really clear separation between the different types of cells.

Details of Technical Method

The technique is a slight modification of those proposed by Rafalko (1946) and Darlington and La Cour (1942).

Air-dried smears of blood or bone-marrow are used and should be fixed as soon as possible, not more than one hour after preparation. The bone-marrow is obtained by sternal biopsy. The fixing solution is composed of: methyl alcohol 15 parts, 5 per cent acetic acid 5 parts, formalin (40 per cent formaldehyde) 1 part, and water 5 parts. The smears are immersed in this fixative for 10 minutes and then treated as follows:

1. Wash in tap water for 10 or 20 minutes.
2. Wash in distilled water for 2 minutes.
3. Place in N/HCl at room temperature for 2 minutes, then (4) in N/HCl at 60° C. for 8 or 10 minutes, and (5) rinse with N/HCl at room temperature.
6. Rinse with distilled water.
7. Stain with leucobasic fuchsin solution (*vide infra*) for 1½ to 2 hours.
8. Put through two or three changes of SO_2 water (*vide infra*) for 1 or 2 minutes.
9. Wash in tap water for 10 to 15 minutes.
10. Then dehydrate by immersing in 40 per cent. 80 per cent, and finally absolute alcohol for 5 minutes each.
11. Clear with xylol.
12. Mount in a neutral synthetic mounting medium.

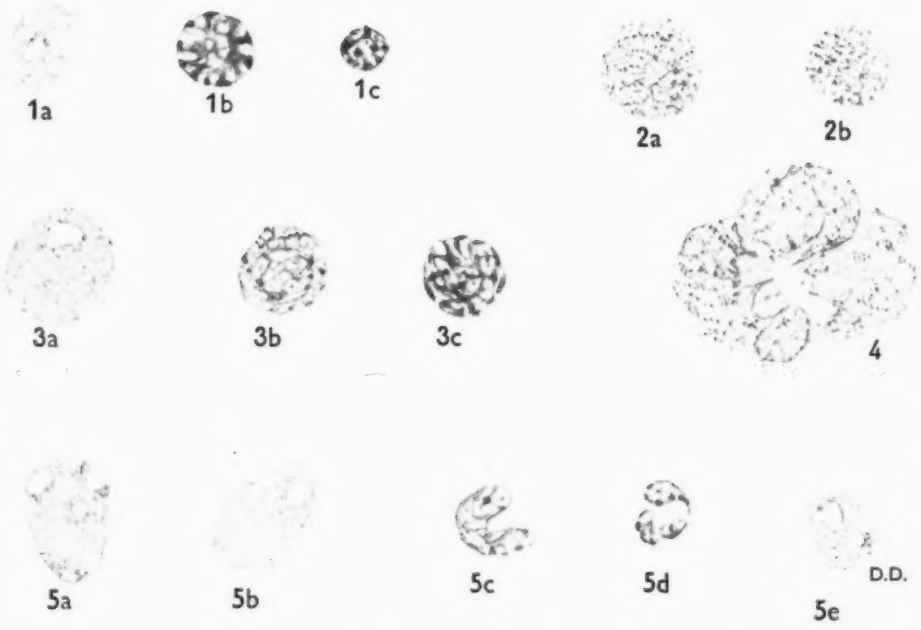
The leucobasic fuchsin is prepared as follows: a 0.5 per cent solution of basic fuchsin is decolorized by bubbling SO_2 through it for 1 hour; if decolorization is not then complete, activated charcoal (1 g. to 100 ml.) should be added to it, and it is shaken and filtered.

The SO_2 water is prepared by bubbling SO_2 from a syphon through distilled water until a saturated solution is obtained.

It is sometimes helpful to counterstain with Jenner's stain to outline the cytoplasm and stain cytoplasmic granules. This can be done without interfering with the Feulgen stain. The Jenner's stain then replaces stage 10 of the technique.

Nuclear Appearances of Blood Cells when Stained by Feulgen's Reaction

With this stain the erythrocytes disappear; the leucocytes and erythroblasts take up stain according to the concentration of desoxyribonucleic acid present in the nucleus; immature cells have a relatively pale coloration, mature cells a deep tint; the cytoplasm does not stain. The pale-stained cells are said to be Feulgen-negative, and the deeply-stained cells Feulgen-positive. Nucleoli show up well as clear spaces in the nucleus with a



BONE-MARROW CELLS STAINED BY THE FEULGEN TECHNIQUE

1.—Lymphocytes from a case of chronic lymphatic leukaemia : (a) Lymphoblast, Feulgen-negative, fine chromatin pattern, two nucleoli ; (b) Immature lymphocyte, Feulgen-positive, but shrunken nucleoli still present; (c) Small mature lymphocyte, Feulgen-positive, with chromatin concentrations round the periphery.

2.—Megaloblasts, untreated pernicious anaemia : (a) early megaloblast—note network arrangement of chromatin and absence of nucleoli; (b) intermediate megaloblast, coarser pattern but network still distinct.

3.—Normal erythroblasts : (a) Pro-erythroblast, Feulgen-negative ; note large nucleolus and fine but distinct chromatin pattern ; (b) early normoblast, moderate Feulgen reaction ; note coarse concentration of chromatin and absence of nucleoli ; (c) intermediate normoblast, large size, Feulgen-positive with lumpy chromatin pattern.

4.—Megakaryocyte, mature cell moderately Feulgen-positive; no nucleoli.

5.—Granular leucocyte series from chronic myeloid leukaemia : (a) typical myeloblast, Feulgen-negative, four nucleoli ; (b) pro-myelocyte, still Feulgen-negative, only one nucleolus; (c) metamyelocyte, still only moderate Feulgen staining ; nucleoli have disappeared ; (d) mature polymorphonuclear granulocyte, Feulgen-positive streaks ; (e) micromyeloblast : note relatively pale Feulgen stain, fine chromatin pattern and the presence of nucleoli.

relatively deep staining rim which is more prominent in the more mature cells. The appearances of individual cells are as follows:

Granulocytes.—In the *myeloblast*, the nucleus takes on a pale stain but the chromatin pattern can often be clearly distinguished; there are two to four large nucleoli clearly detectable as colourless spots. The *micromyeloblast* is rather more deeply stained but nucleoli are clear. The *promyelocyte* and *myelocyte* show changes indicating increasing maturity—the nucleoli decrease in number and size and show a thin surrounding layer of Feulgen-positive material. The *metamyelocyte* has no nucleoli, but is not so deeply stained as the mature *polymorphonuclear*, which has Feulgen-positive coarse chromatin with two or three prominent positive spots, usually one in each lobe of the nucleus.

Lymphocytes.—The *lymphoblast* has a Feulgen-negative nucleus of fine structure, not very clearly defined, and there are one or two prominent nucleoli. The *immature lymphocyte* has a coarser and more deeply staining nuclear chromatin, but nucleoli are still present though small. The nucleus of the *mature lymphocyte* is composed of Feulgen-positive blocks of chromatin arranged mainly around the periphery.

Monocytes.—The mature monocyte has a fine chromatin structure and stains a faint pink. No aggregations of chromatin occur, and there are no nucleoli.

Erythroblasts (Nomenclature according to Israëls, 1941).—The *pro-erythroblast* shows a distinct chromatin network but is Fuelgen-negative; nucleoli are prominent and are distinguished by a deeply stained surrounding layer.

The *normoblasts* show early appearance of Feulgen-positive material; even the early stage is quite deeply stained and shows very well the coarsening and clumping of the chromatin strands. The intermediate normoblasts are fully Feulgen-positive and show the typical coarse chromatin structure.

In the *megaloblast* series the fine network nuclear structure shows up very strikingly when this technique is used. The early stages show only moderate Feulgen staining; the nuclear strands of the intermediate stage are fully Feulgen-positive.

Megakaryocytes.—The large nucleus of the mature cell is moderately Feulgen-positive and no nucleoli are present.

Discussion

The Feulgen reaction has thus several uses as a supplementary technique in clinical haematology.

1. It gives a clear decision about the stage of maturity of a given cell. This is very important in the leukaemias. A not uncommon cause of incorrect diagnosis is confusion between small myeloblasts (micromyeloblasts) and lymphocytes. Sometimes with ordinary stains the differentiation is very difficult even with a good technique; in particular, nucleoli are often obscured by Romanowsky stains. Schulten (1937), for example, shows on Table 13 in his Atlas a group of cells that he cannot clearly define as myeloblasts or lymphocytes. The Feulgen stain distinguishes clearly between the Feulgen-negative myeloblasts with well-defined nucleoli and the Feulgen-positive lymphocytes without nucleoli.

In chronic myeloid leukaemia the presence of myeloblasts can be used to assess the progress of a case and response to treatment; if these immature cells are few and their proportion decreases with treatment, the prognosis is good. It is much more difficult to assess a case of lymphatic leuleuamia in this way, because with Jenner-Giemsa stain it is not a simple matter to detect immature lymphocytes. The Feulgen stain provides the necessary information, as the proportion of negative or weakly positive lymphoblasts and immature lymphocytes with detectable nucleoli is readily determined and these cells contrast well with the Feulgen-positive mature lymphocytes without nucleoli.

2. The characteristic difference between normoblasts and megaloblast is in the nuclear structure, the chromatin being arranged respectively in clumps or in network of strands. The Feulgen stain gives a sharper picture of the intimate nuclear structure than is possible with Jenner-Giemsa, and another difference is that the normoblasts become more deeply stained at an earlier stage of development than the megaloblasts.

3. The differentiation between lymphoblasts and myeloblasts is a problem as old as clinical haematology, and many techniques—dark-ground illumination, supravital staining, oxidase stains—have been applied with indifferent success. The Feulgen reaction helps because it defines nucleoli. Naegeli (1923) pointed out that, whereas lymphoblasts have few nucleoli, in myeloblasts they are usually multiple. In our experience there are usually one or two nucleoli in lymphoblasts, rarely three; whereas in myeloblasts there are two to four nucleoli, most commonly three. Nucleolus-counting can be a difficult task with Jenner-Giemsa-stained preparations; with Feulgen stain it is an easy matter to see whether the majority of the immature cells have few or many nucleoli. The presence of a majority of cells with three or

more nucleoli suggests a myeloblastic form of leukaemia.

The nucleoli with their rim of dark-staining material are a typical feature of the Feulgen-stained cells. They appear often much larger than in the Jenner-Giemsa stained preparations. Thorell has related the number and size of the nucleoli to the amount of cytoplasm that is to be formed by the cell in the course of development. The myeloblasts of acute leukaemia often have numerous and prominent nucleoli, yet fail to develop cytoplasm. Two possible explanations are that the cells are abnormal, like malignant cells, and do not develop normally; or that they are normal cells in an abnormal environment. In support of the latter explanation are Israëls' (1940) observation that leukaemic myeloblasts in culture could mature into myelocytes, and Schwind's (1947) claim that injection of normal blood plasma tends to decrease the proportion of myeloblasts in the blood. It is therefore likely that in pathological states the connexion between nucleolar mass and cytoplasm formation is disturbed.

Summary

1. Details are given of a rapid technique for applying the Feulgen reaction to blood and bone-marrow cells.

2. The appearances of the more important blood and bone-marrow cells when stained by this Feulgen technique are described and illustrated.

3. The Feulgen reaction is a valuable supplementary technique in clinical haematology for the following reasons:

(a) It distinguishes between immature and mature cells, especially between lymphocytes and micromyeloblasts, and it can be used to assess the proportion of immature cells in lymphatic leukaemia.

(b) The nuclear differences between normoblasts and megaloblasts are sharply defined.

(c) It defines nucleoli very clearly and can therefore aid in distinguishing lymphoblasts with one or two nucleoli from myeloblasts with two to four nucleoli.

4. The suggested correlation between number and size of nucleoli and subsequent cytoplasm formation appears to be disturbed in disease.

The plate was painted by Miss D. Davison, medical artist to the University.

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HAEMOSTASIS WITH AN EASILY PREPARED STABLE THROMBIN SOLUTION

BY

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During physiological blood coagulation after injury the "clotting enzyme," thrombin, is always formed in excess. Such large amounts of liberated thrombin would ultimately cause extensive intravascular thrombosis, were it not for the presence of substances with antithrombic activity, which destroy the thrombin formed, partly before and partly after its action upon fibrinogen. While these substances are of great protective importance in physiological blood coagulation, they have hampered *in vitro* work considerably. Much difficulty has been caused by them in the quantitative determination of prothrombin, because it has not been possible to convert prothrombin to thrombin completely in plasma or whole blood and to measure thereafter the activity of the latter in clotting fibrinogen. An undeterminable part of the thrombin formed has always been destroyed by antithrombic activity.

In the same way, the *in vitro* isolation of thrombin for the purpose of haemostasis has been hampered by such destruction of thrombin immediately after its formation. While American authors (Cohn and others, 1940, 1946; Edsall and others, 1944; Milstone, 1942; Seegers, 1940; Seegers and McGinty, 1942; Seegers and others, 1938, 1945) have solved this problem by the isolation, instead of thrombin, of its precursor, prothrombin, and by subsequent elaborate purification of the latter to separate it from as much antithrombin as possible, yet they have not been able to remove all the antithrombin, and this is shown by the fact that their preparations are not very stable in the liquid state.

Recently it has been found that the antithrombic activity of plasma can be specifically suppressed by dilute ethyl alcohol (Sternberger, 1947), and the effect was made use of for the development of a simple two-stage method for the determination of prothrombin that is independent of any inactivators and that is thus able to determine prothrombin quantitatively (Sternberger, 1947, 1948). In the present communication preliminary experiments are reported upon the isolation and clinical use of thrombin from blood in which the antithrombic activity has been suppressed and prothrombin subsequently converted to thrombin. It will be shown that this preparation offers the following advantages over previous ones:

1. The thrombin obtained is completely free from antithrombin, not only separated from it by fractional purification as in previous methods. Therefore the thrombin is stable for many months, in the liquid state, diluted or undiluted. Other thrombin preparations are not stable once they have been diluted (Bailey, 1945; Glazko, 1947).

2. No elaborate purifications are necessary in the preparation of our thrombin. The procedure is, therefore, so simple that any laboratory can produce its own thrombin for surgical haemostasis. No complicated equipment is necessary.

3. During its preparation our thrombin is coprecipitated with acacia. The presence of acacia gives the thrombin a sticky consistency. This makes it possible to use thrombin solutions effectively with much smaller activity than hitherto, derived from a correspondingly smaller amount of blood.

Method of Preparation of Thrombin

Step 1: "Thrombinization."—A temperature between 16° and 22° C. (60.8° to 71.6° F.) is maintained while the following ingredients are placed

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successively into a flask and stirred after each addition:

380 parts of 50 per cent by volume of ethyl alcohol in normal saline solution.

145 parts of normal saline solution.

25 parts of 0.2 M calcium chloride solution.

210 parts of human blood (whole blood ; 9 parts of blood obtained by venepuncture and rendered incoagulable by addition to 1 part of 0.1 M sodium oxalate solution).

75 parts of human milk.*

75 parts of 50 per cent by volume of ethyl alcohol in normal saline solution.

The material obtained after the lapse of about 5 to 10 minutes (crude thrombin) will clot an equal volume of human plasma in 6 to 8 seconds. It is very stable. It may be processed immediately or may be kept in the refrigerator without loss of activity for at least eight months.

The subsequent steps may be performed at a temperature between 16° and 28° C. (60.8° to 82.4° F.).

Step 2: Denaturation of labile blood proteins.—To 32 parts of "crude thrombin" (shaken well to obtain a uniform suspension) are added 18 parts of 95 per cent ethyl alcohol by volume. The whole is shaken violently and centrifuged immediately. The supernatant fluid is decanted, and Step 3 immediately proceeded with.

Step 3: Elution.—The sediment, stirred well to break it up as completely as possible, is again dissolved in 32 parts of a 10 per cent solution of powdered acacia in normal saline and centrifuged.

Step 4: Co-precipitation.—To 30 parts of the supernatant fluid are added 32 parts of 95 per cent of ethyl alcohol by volume and the whole is well mixed and centrifuged. The supernatant fluid is decanted and the next step immediately proceeded with.

Step 5: Resolution.—The sediment is dissolved in 3 parts of normal saline solution. This is done by wiping the sticky sediment around a glass rod and breaking it up by pressing it at the same time against the walls of the centrifuge tube. It will then dissolve easily in the relatively small volume of added liquid.

We usually dissolved the sediment of thrombin and acacia in a solution of 1:1,000 merthiolate (sodium ethyl mercurithiosalicylate) in normal saline. When thrombin was being prepared for surgical use, sterile technique was employed from the moment of the second addition of alcohol on (in Step 4) (sterilization by 50 per cent alcohol). In this case the final thrombin and acacia solution was dissolved in 1:7,500 merthiolate in normal saline solution. All our thrombins so prepared were tested for sterility, and in none did any growth take place.

* Many samples of blood carry with them enough thromboplastin for making it unnecessary to add further thromboplastin for the conversion of prothrombin to thrombin in the presence of ethyl alcohol. But the occurrence of sufficient amounts of thromboplastin in the blood is very irregular, so that it becomes necessary to use additional thromboplastin to obtain a dependable preparation of thrombin.

In this preparation of thrombin from human blood *human* milk is used. It has been claimed that foreign protein in thrombin preparations would sensitize the patient. However, conclusive proof of this warning is lacking.

Cow's milk also contains thromboplastin, but its activity is much smaller than that of human milk.

Results

The Table gives the clotting times obtained with our acacia-containing thrombin solution. No loss of activity of the thrombin solution took place during a period of six months' storage in the ice box.

TABLE

CLOTTING TIME WITH ACACIA-CONTAINING THROMBIN SOLUTION

Dilution of thrombin (after addition to test plasma)	Clotting time (seconds) (assay by adding 0.2 ml. of diluted thrombin to 0.2 ml. of oxalated human plasma)
1 : 2	2 1/2
1 : 6	2
1 : 10	5 1/2
1 : 20	10
1 : 40	18

Clinical Use

Because of the admixture with acacia, the thrombin preparation, when applied to a wound, will adhere to the bleeding surface. It does, therefore, as shown by clinical experience, prove effective as a haemostatic agent, although its *in vitro* activity is less than that of some other preparations of thrombin, this corresponding to the smaller amount of blood required for its isolation.

Our thrombin can be applied by simply placing a few drops of the gelatinous solution upon a plug of gauze. The stickiness of the acacia prevents the thrombin from being soaked up by the gauze, the solution tending to adhere to its surface. Thus it produces an effective haemostatic layer between gauze and bleeding wound. The fact that the preparation is not soaked up by the gauze also adds to the economy of its use. The gauze covered with thrombin-acacia is applied to the wound until haemostasis is produced. It may then be removed. While a clot is produced at the interface between the thrombin-acacia and the bleeding surface, this

clot—because of the stickiness of the preparation —does not extend beneath the surface of the gauze, and does not, therefore, cause fresh laceration and fresh bleeding when it is subsequently removed (a difficulty encountered by Tidrick and others (1943) in the use of their preparation with cotton plugs and bits of gauze). Bleeding also recurs, as reported by Bailey and others (1945), if fibrin foam (Cohn and others, 1940, 1946 ; Edsall and others, 1944) applied to a wound with thrombin is removed after production of haemostasis.

The stability of our preparation in the liquid form makes it possible to use a few drops of thrombin for one purpose, and to store the remainder of the bottle in the refrigerator until new requirements arise. This further adds to the economy of the preparation, in contrast to those products that are supplied in a powdered form and cannot be stored after once having been dissolved. Even a few hours' storage at subtropical room temperature (26° to 28° C. (78.8° to 82.4° F.) in our laboratory and hospital at the time this preparation was being developed and tried clinically) does not impair the effectiveness of our preparation. The thrombin prepared by Cohn and his team is rapidly destroyed at temperatures higher than room temperature (Bailey and others, 1945).

The thrombin described here has been used by Dr. Lachmann, Chief of the Otolaryngology Department of this hospital. He reports as follows:

"The thrombin was used in twenty tonsillectomies and in eight Caldwell-Luc's operations on the maxillary sinus. In tonsillectomies, at the completion of the operation a tampon of gauze covered with a few drops of thrombin solution was applied with slight pressure for 1 to 2 minutes to the tonsillar bed. It was then removed. At this time the wound was dry in every instance, including cases in which bleeding before application of thrombin was very strong. Only spurting blood vessels were ligated before using the thrombin. Many cases would have required careful ligation, had thrombin not been used.

In Caldwell-Luc's operation on the maxillary sinus, 1 to 3 ml. of thrombin were instilled upon completion of the operation. Bleeding always stopped within a short time (2 to 5 minutes). The cavity remained without tampon. Only in one case, which showed very heavy haemorrhage because of hypertension (270/140 mm. Hg), the cavity was packed lightly with gauze upon which thrombin had been placed. This stopped the haemorrhage at once, without recurrence upon removal of the tampon the following day."

The thrombin was also used in a case of dicoumarol hypoprothrombinemia (Sternberger, 1948) (prothrombin: 12.5 per cent determined by the stabilized thrombin method (Sternberger, 1947)). The patient started to bleed from the operation wound eleven days after the second stage of Lahey's abdominal-perineal resection of the rectum (Dr. Joseph). Haemostasis with thrombin was immediate.

Summary

The principle of the suppression of the antithrombic activity of plasma by ethyl alcohol was applied to the development of a new method for the isolation of thrombin. The simplicity of the method makes it possible for any clinical laboratory to prepare thrombin for its own use for surgical haemostasis. The thrombin prepared is stable in the liquid form. The clinical use of the preparation in haemorrhage and the advantages of admixture of acacia with thrombin have been discussed.

I wish to thank Prof. E. Wertheimer, Chief of the Department of Pathological Physiology, Hebrew University, and Director of the Chemical Laboratory, Rothschild Hadassah University Hospital, for his unfailing counsel during this investigation. I am grateful to Dr. Lachmann, Chief of the Otolaryngological Department, Rothschild Hadassah University Hospital, for his readiness to make the first clinical trials with the preparation described, and for his advice during its surgical application.

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SEROLOGICAL ASPECTS OF WEIL'S DISEASE

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The agglutination test for Weil's disease (leptospirosis icterohaemorrhagica) is essentially the same as other serological procedures. The disease, however, is comparatively uncommon, and the leptospira suspensions are relatively unstable. For these reasons there has been a tendency for work on leptospirosis to be concentrated in a few laboratories. The observations recorded here are based on the findings in agglutination tests carried out on serum from 219 cases of leptospirosis and from 875 patients suffering from other diseases. Epidemiological and clinical notes on some of these cases have been described by Broom and Alston (1948).

Serological Methods

Agglutination of formalinized cultures of *Leptospira icterohaemorrhagiae* was the diagnostic test used in this work. Some workers prefer suspensions of living organisms, but the need to have well-grown, active cultures always available is a disadvantage in routine work, and in our experience the two methods are equally sensitive. Furthermore, with either fresh or inactivated serum, formalinized suspensions agglutinate in the same manner over the whole titre, whereas living cultures lyse in some dilutions of serum, and thereafter present a series of varying agglutination pictures at the different serum-concentrations. In addition, a single strain of leptospira may be lysed by one serum and agglutinated by another in the same dilutions. All these variations are of interest and value for research purposes, but they introduce unnecessary complications for clinical diagnosis.

Antigen.—The same strain of *L. icterohaemorrhagiae* was used throughout, although parallel tests were often carried out with other strains. This strain, "Jackson," was isolated in 1935 from a human case of Weil's disease, and contains both the A and B antigens described by Petersen (1939). Though now completely non-virulent for guinea-pigs, it provides a satisfactory agglutinating suspension.

Stock cultures are maintained in Fletcher's (1927) agar medium and are used to inoculate flasks of Fletcher's broth (in which broth is substituted for agar) or of Korthof's (1932) medium. The cultures are incubated at 26° C. for five to seven days until they reach a density of about 10^7 leptospirae per ml. In practice, no count is made; the density is judged by examinining a drop of culture by dark-field illumination with a 16-mm. objective and a × 10 ocular. Sufficient formalin is added to give a final concentration of 0.2 per cent; the suspension is centrifugalized, and any deposit is discarded.

The stability of formalinized suspensions of leptospirae is very variable, and the responsible factors are not yet known. It is our custom, therefore, to prepare quantities of only 50 or 60 ml. at one time, and to renew the suspensions at intervals of a few weeks. The suitability of the new suspension is determined by testing the titres of known positive sera in parallel against old and new suspensions. If the new culture differs in agglutinability to an appreciable extent, it is discarded. No attempt has been made to work out a factor for different suspensions, as is done, for example, with standard agglutinating suspensions of the enteric group of bacteria.

Agglutination test.—The technique used is that described by Schüffner and Mochtar (1927). Mixtures of serum and antigen are prepared so that the final dilutions of serum are 1/10, 1/30, 1/100, 1/300, etc., and interaction is allowed to continue overnight at room temperature or at 5° C. A single drop of each dilution is examined, without a coverglass, by dark-ground illumination, using a 16-mm. objective and a × 12.5 ocular. In our opinion this is the best method of reading the result, because this magnification allows true agglutination in low dilutions (1/10 and 1/30) to be distinguished from the non-specific clumping which is produced by a small proportion of sera. In true agglutination, loose floccules of leptospirae are seen

against a background containing no free organisms, whereas non-specific clumping shows small, compact, refractile masses in which only a proportion of the leptospirae have become entangled, leaving the majority free in the field. For confirmation in doubtful cases the specimen is examined with a 2-mm. objective.

Specificity of the Reaction

Most observers would agree with Smith and Davidson (1936) that " the sero-reaction is entirely specific, agglutinins and lysins being formed as a result of leptospira infection only." These observers found no agglutination in serum dilutions of 1/10 in a series of 403 control tests. Stuart (1946) states that he has " never found antibodies at a titre of 1/10 in people who had not been exposed to known or highly probable leptospiral infection." Ward and Turner (1942), in a survey of workers in different trades, accept a titre of 1/10 as evidence of old infection.

Evidence of a more direct nature is available from the work of Alston and Brown (1935), who tested sera from sewer-workers and found that samples with agglutination titres of 1/100 would protect guinea-pigs against infection with virulent *L. icterohaemorrhagiae*. The subjects were in normal health when the blood samples were taken, and the agglutination was presumed to be due to residual antibodies from past infection. Similar results were reported by Mason (1938), who obtained partial protection by serum showing a titre of 1/30, and complete protection by sera with titres of 1/100 and 1/300. Our experience of the agglutination test is, in the main, in agreement with these opinions. In this series there were 875 patients where the final diagnosis was not Weil's disease. Of these, nine sera gave agglutination in dilutions up to 1/30, and a further fourteen in a dilution of 1/10 only. Most of these patients worked at occupations where Weil's disease is a recognized hazard, for example on farms, in coal mines, or in sewers, so the possibility of previous contact with leptospirae could not be ruled out.

Para-agglutinins

Although a positive agglutination test is a specific sign of infection with leptospirae, there is some serological overlap between *L. icterohaemorrhagiae* and the dog leptospira, *L. canicola*, which also is infective to man though only to a minor extent. In most instances the titre of a serum is 10 to 100 times higher for the homologous species, but occasionally, in the early stages of the disease, the titres run parallel, or may even be higher with *L. canicola*. According to Gispen and Schüffner (1939) this is more likely to happen when the infecting leptospira is of the " incomplete " type of Petersen. An example of this effect is shown in Table I, which sets forth the titres against the two leptospirae of specimens of serum taken on the sixteenth and twenty-sixth days of illness. An earlier specimen, of the eighth day, was negative with both leptospirae. Absorption tests on the sample of the sixteenth day showed the infection to be due to *L. icterohaemorrhagiae*, but the strain was not isolated to test whether it was of the incomplete type.

Time of Appearance of Antibodies and Titres Reached

A certain number of observations have previously been made on the interval which elapses between the onset of symptoms and the appearance of demonstrable agglutinins in the serum, and also on the manner in which the antibody titre rises during the course of the disease. In the opinion of Postmus (1933) no positive result is to be expected until after the sixth day; reactions then occur in low dilutions (1/10 to 1/25), and

TABLE I

AGGLUTINATION OF *L. ICTEROHAEMORRHAGIAE* AND *L. CANICOLA* WITH WEIL'S DISEASE SERUM

	Dilution of serum							
	1/10	1/30	1/100	1/300	1/1000	1/3000	1/10,000	
L. icterohaemorrhagiae ..	+	+	+	+	+	±	—	Serum of 16th day
L. canicola	+	+	+	+	+	+	—	
L. icterohaemorrhagiae ..	+	+	+	+	+	+	—	Serum of 26th day
L. canicola	+	+	±	—	—	—	—	

+ = agglutination; ± = partial agglutination; — = no agglutination.

TABLE II

RELATION BETWEEN DAY OF DISEASE AND APPEARANCE AND DEVELOPMENT OF ANTIBODIES

Day of disease									
2	3	4	5	6	7	8	9–13	14–20	<20
0							300		
	0					300			
	0						1,000		
		0				100		300	
		30						10,000	
			0					300	1,000
			0					300	3,000
			0	0	10		1,000	3,000	
			0				1,000	3,000	
			30				1,000		
			100				3,000		
			300					3,000	
				0					1,000
				0					3,000
				0					3,000
				0			300		10,000
				10			1,000		
				30			100		
				30			3,000		
				30				3,000	
				30			1,000		30,000
					0			1,000	
					0			1,000	
					10			300	3,000
					30				10,000
					100		300	1,000	3,000
					100			3,000	3,000
					300			3,000	3,000
					1,000			3,000	
						0		3,000	30,000
						30	1,000		
						30		3,000	
						100		1,000	
						100		3,000	
						100			10,000
						300		1,000	
						300	1,000	3,000	
				0			0	100	300
			0				0		30,000

0 = No agglutination at dilution of 1/10. 10, 30, etc. = Reciprocals of agglutination titres.

the titre rapidly rises to reach a maximum of 1/10,000 to 1/100,000 by the end of the third week. Kisker (1935) considers that agglutinins are not present before the eighth day, when titres of 1/160 to 1/300 are obtained; thereafter a maximum of 1/40,000 to 1/80,000 is reached between the fifth and seventh weeks. Mochtar and de Reede (1941) also found the agglutination test negative during the first week; the highest titres in their series occurred during the third and fourth weeks.

We have carried out tests at the relevant times on thirty-nine patients, and the results are set out in Table II. The wide variation in the day on which antibodies appear, the rate at which the titre rises, and the maximum reached, is very striking. The earliest positive agglutination was recorded on the fourth day after onset, but negative as well as positive results were obtained on all days up to the eighth, and two sera were still negative on the twelfth and thirteenth days respectively. In some instances, of course, the onset may have been gradual and the stated day may not be quite accurate, but the disease began abruptly in the patient whose serum gave a positive result on the fourth day.

As will also be seen from the table, there is no apparent correlation, either positive or negative, between the time of first appearance of antibodies and the final titre. On the average, the titres seem to be lower than those often recorded, and we have never found anything approaching, for instance, the 1/2,000,000 reported by Senthille and others (1946). We have tested serum from 169 patients during the third week of illness or later, and the highest titres reached are shown in Table III. Only about 20 per cent rose to 1/10,000, and 7 per cent as low as 1/300.

TABLE III

HIGHEST TITRE OF 169 SERA TESTED DURING THIRD OR LATER WEEK OF DISEASE

	Reciprocal of titre				
	300	1,000	3,000	10,000	30,000
No. of sera with titre ..	12	47	73	30	7

Effect of Therapeutic Serum

In some cases the question arises whether the administration of therapeutic serum has vitiated the result of a test. Alston (1940) found that the agglutination became negative in rabbits within three days after the injection of 10 ml. of anti-leptospiral serum per litre of plasma. We tested the result in the human case of influenza, described by Robertson (1946), who received approximately 40 ml. of immune horse-serum per litre of plasma. The agglutination titre of the horse serum was 1/30,000, which would give a calculated titre of about 1/1,000 in the general circulation. Samples of blood taken five and sixty minutes after administration showed a titre of 1/300. The level fell to 1/100 after twelve hours, and to 1/30 after twenty-four hours.

Persistence of Antibodies

It has been shown by a number of workers that antibodies can be demonstrated in the serum many years after recovery from an attack of Weil's disease. Thus Kisker (1935) found positive agglutinations from two to sixteen years after recovery; Uhlenhuth and Fromme (1930) after twenty-two years; and Stuart (1939) after twenty-eight years. We have had few opportunities to examine serum after recovery, and the longest interval was four years, but agglutinins were present in all cases. The practical importance of these residual antibodies will be referred to later.

Discussion

A number of factors must be taken into account when the clinical significance of the results of leptospiral agglutination tests is being assessed. Although in general the test is positive about the end of the first week, there are occasions when the appearance of agglutinins is delayed, as in the two cases cited above which still reacted negatively on the twelfth and thirteenth days respectively. A negative finding may thus merely mean that the blood was examined before antibodies were present. Multiple negative tests throughout the course of illness will exclude Weil's disease with considerable certainty, because it must be very rare to find cases such as that reported by Garnier and Reilly (1917), in which, although leptospirae were isolated from the patient, no antibodies appeared in the blood.

Positive agglutination reactions may be the result of (1) the administration of immune serum; (2) the presence of residual antibodies from past infection; (3) present disease.

The giving of serum might affect the result of the test in the early stages, and should be reported by the physician who sends blood for examination.

The possibility of past infection is important in patients who have been in contact directly or indirectly with rats. McKeon and Brown (1936) describe an attack of infective hepatitis in a miner, where the diagnosis was complicated by a positive leptospira agglutination. Three similar cases have occurred in this series. The patients, a farm worker, a seaman, and a refuse collector, all suffered from jaundice. Each case showed a positive agglutination titre of 1/100 or 1/300 but, as there was no increase at subsequent tests, the illness was obviously not Weil's disease.

When the clinical picture is suggestive, the results shown in Table III indicate that a positive agglutination in serum dilutions of 1/1,000 or more may be accepted as confirming the diagnosis. The interpretation of a lower titre is difficult if the findings of only one test are available. As is shown in Table II, titres as low as 1/300 or even 1/100 may occur quite late in the disease. In two of these patients the diagnosis was proved correct by isolating leptospirae from the urine. Low readings cannot therefore be automatically regarded as resulting from past infection. The correct decision can be reached only by repeated examinations.

Early in the disease, say up to the fourteenth day, we would consider positive agglutination even to the lowest titres to be suspicious, and recommend the testing of further specimens.

Summary

1. A description is given of the material and methods used in applying the leptospira agglutination test to serum from 219 cases of Weil's disease and 875 of other illnesses.

2. Evidence is put forward confirming the specificity of the test.

3. The shortest interval after the onset of symptoms at which antibodies were detected in the serum was four days. Both positive and negative reactions occurred up to the eighth day ; two sera which later became positive were still negative on the twelfth and thirteenth days respectively.

4. Examples are given of the variability of the rate of production of antibodies, and of the highest titre reached in individual patients.

5. In a single experiment samples of serum were tested from a subject who had received 40 ml. of antileptospira serum per litre of plasma. From an initial titre of 1/300 at five minutes, the antibodies fell to 1/30 at twenty-four hours.

6. In discussing the clinical interpretation of agglutination tests, stress is laid on the need to consider the result in conjunction with the length of time after infection, and to bear in mind the possibility of previous contact with infection.

7. With suggestive clinical symptoms, an agglutination titre of 1/1,000 is considered to confirm the diagnosis. With lower titres and in doubtful cases repeated tests are recommended.

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SALMONELLA BIRKENHEAD: A NEW SALMONELLA TYPE CAUSING FOOD-POISONING IN MAN

BY

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During the six weeks from mid-July to the end of August, 1947, nine strains of a new *Salmonella* type were received from nine patients living in different parts of England, five of them in the Cambridge-Ipswich area.

Clinical History

Of the nine cases, seven gave a history typical of acute gastro-enteritis; one had enteritis only, and the last patient, who was investigated for reasons not relevant to the present paper, was a symptomless excretor of the organism. The patients with gastro-enteritis complained of sudden onset of the disease with headache, abdominal pain, vomiting, and diarrhoea, and all showed some rise in temperature. The eighth case, a child, had similar symptoms except that she never vomited. Three patients were sufficiently ill to be admitted to hospital, one of them being described by her doctor as having symptoms suggestive of enteric fever. A fourth patient had been in hospital for six weeks with cardiac disease when he suddenly complained of diarrhoea; this case terminated fatally after a two-day illness, but it is impossible to say how far the *Salmonella* infection may have contributed to the fatal issue. The findings are summarized in Table I.

In all nine cases the organism (*Salm. birkenhead*, VI, VII; c ⟷ 1,6) was isolated from the faeces. An attempt was made to determine whether any antibodies specific to the new *Salmonella* were present in the patient's serum on recovery. Sera obtained from four cases gave the results shown in Table II. It will be seen that one case only, M.B., gave a high titre to the infecting organism. Such a finding is not uncommon in cases of infection

TABLE II

EXAMINATION OF SAMPLES OF BLOOD SERUM FROM PATIENTS

Case	Agglutination titre to *Salm. birkenhead*		
	0	H (c)	H (1,6)
J.B.	1:25	—	—
M.B.	1:25	1:320	1:640
A.G.	—	—	1:100
S.	1:50	—	1:50

All sera taken about four weeks after onset.

TABLE I

CLINICAL DATA AND DISTRIBUTION OF PATIENTS

Case	Age (years)	Symptoms	Duration of illness	Domicile	Date of onset
T.G.	46	G.E.	2 days (fatal)	London, Middx.	18.7.47
H.G.	Adult	—	—	Blunham, Beds.	15.8.47
J.B.	64	G.E.	27 days	Birkenhead, Lancs.	2.8.47
J.L.	2½	E.	7 days	Ipswich, Suffolk	18.8.47
E.A.	35	G.E.	15 days+	Blunham, Beds.	11.8.47
M.B.	8	G.E.	7 days	Soham, Cambs.	25.8.47
A.G.	3	G.E.	9 days	Ipswich, Suffolk	29.8.47
S.	Adult	G.E.	7 days	Swindon, Wilts.	end of Aug. 47
L.	Adult	G.E.	7 days+	Newquay, Cornwall	25.8.47

G.E. = gastro-enteritis. E. = enteritis. — = no symptoms. + = patient not recovered when last seen.

with members of the *Salmonella* group, in which a rise in antibody titre is much more variable than in enteric infection.

Epidemiology

Since all the nine cases described occurred during a six-week period, it was thought that some article of diet, such as a new type of food, might be responsible for this infection. Home visits were paid early in October, 1947, to the five persons in the Cambridge-Suffolk area. Inquiries were made into any unusual food eaten by the patients, into the local sources of food, into visits by patients to other places, and into visitors to the household during the three weeks before onset. No lead was obtained to suggest that further investigation would determine the source of infection. In one instance (A.G.), there was another case of gastro-enteritis in the household which preceded the case described (A.G.) by thirty hours; this patient—a child—was not investigated.

Bacteriology

On MacConkey agar the Gram-negative motile bacillus isolated from the faeces of the cases described gave typical *Salmonella* colonies about 3 mm. in diameter, round, with slightly crenated edge and glistening surface. The biochemical reactions were as follows: glucose, maltose, mannitol, dulcitol, sorbitol, rhamnose, xylose, and trehalose were fermented with the production of acid and gas in twenty-four hours, and arabinose in three days. Lactose, saccharose, salicin, adonitol, dextrin, and inositol were not fermented within three weeks. Gelatin was not liquefied, indole was not produced, and urea was not broken down. The Voges-Proskauer test was negative, methyl red test positive, hydrogen sulphide was produced in lead acetate agar, and the citrate utilization test was positive.

All nine strains gave identical serological reactions in that they were agglutinated to titre by *Salm. oranienburg* "O" serum. Reciprocal absorption tests by a heterologous strain removed all the homologous agglutinins. Thus the somatic antigenic complex is VI, VII. Of the nine strains, five were agglutinated to titre by *Salm. paratyphi C* "H" (c) serum, two to titre by *Salm. anatum* "H" (1,6) serum, and two to titre by both these sera. On growing the five strains in "H" (c) phase in sloppy agar containing antiserum *Salm. paratyphi C* "H" (c) a second phase "H" (1,6) was isolated. Similarly on growing the two strains in "H" (1,6) phase in sloppy agar containing *Salm. newport* var. *puerto rico* "H" (1,2,3) serum, the first phase "H" (c) was isolated. Reciprocal absorption of *Salm. paratyphi C* "H" (c) serum with the first phase of the new organism and a serum made against the new organism with *Salm. paratyphi C* "H" (c) completely removed all the homologous agglutinins. Similarly reciprocal absorption tests using *Salm. anatum* "H" (1,6) serum and an antiserum to the new strain proved these two phases to be identical. The flagellar antigens are therefore represented as c ⟷ 1,6.

Discussion

The main interest in this new organism, *Salm. birkenhead* (VI, VII; c ⟷ 1,6) lies in its close serological relationship to *Salm. paratyphi C* (VI, VII [Vi]; c ⟷ 1,5), *Salm. cholerae-suis* (VI, VII; c ⟷ 1,5) and *Salm. cholerae-suis* var. *kunzendorf* (VI, VII; c ⟷ 1,5).

Salm. paratyphi C may contain the Vi antigen found in many strains of *Salm. typhi* and *Salm. ballerup*; this antigen was absent from all nine strains of *Salm. birkenhead*, so in this respect the somatic complex differed from many but not all strains of *Salm. paratyphi C*. *Salm. cholerae-suis* and the kunzendorf variety both have the same somatic complex as *Salm. birkenhead*. It is well known that the flagellar antigen c can be obtained from the kunzendorf variety of *Salm. cholerae-suis* (Gard, 1937; Bruner and Edwards, 1939); therefore serologically the only constant difference between *Salm. paratyphi C*, *Salm. cholerae-suis*, *Salm. cholerae-suis* var. *kunzendorf*, and *Salm. birkenhead* is that the second phase of the first three is 1,5, of the last 1,6.

The diagnostic biochemical reactions of *Salm. paratyphi C*, *Salm. cholerae-suis*, the kunzendorf variety, and *Salm. birkenhead* are given in Table III. From this it will be seen that the reactions of *Salm. paratyphi C* and *Salm. birkenhead* are very similar.

TABLE III

COMPARISON OF DIFFERENTIAL BIOCHEMICAL REACTIONS

	Arabinose	Dulcitol	Rhamnose	Trehalose	H_2S
Salm. paratyphi C	−+$_1$	+$_1$	+ (1-2)	+ (2-3)	+
Salm. cholerae-suis	−	∓	+$_1$	−	−
Salm. cholerae-suis var. *kunzendorf* ..	−	∓	+$_1$	−	+
Salm. birkenhead	+$_2$	+$_1$	+$_1$	+$_1$	+

− negative. +$_1$ acid and gas in 1 day. ∓ negative or slight positive.

In spite of the close relationship between the four organisms discussed, the clinical picture is very different. *Salm. paratyphi C* infection normally produces a serious illness, usually with continued fever. Complications of a septic type are not uncommon sequelae (Topley and Wilson, 1946), and two fatal cases of meningitis due to this organism have been described (Beattie and others, 1946).

Salm. cholerae-suis and the kunzendorf variety also cause serious disease. Harvey (1937) states that in sporadic cases in America the case-fatality rate in patients under 25 years of age is 19 per cent, in patients over 25 years of age 58 per cent. In his review of the literature describing epidemics due to this organism, he finds only five fatal cases among the 1,425 mentioned. Patients not uncommonly have sequelae such as pulmonary, bone, and joint infections, and endocarditis. He also describes four fatal cases of meningitis. In this country there have been few cases of this infection ; twelve were studied by Scott (Reports 1923 to 1939). During this period there were in addition four examples of generalized infection (Nabarro and others, 1929, two cases ; Boycott and McNee, 1936, one case ; Guthrie, 1941, one case). In this laboratory seven strains were studied between 1940 and 1947 ; two of these caused fatal septicaemia (Schwabacher and others, 1943): of the remaining five, three were isolated from blood cultures, two from faeces: all five patients recovered.

Salm. birkenhead caused clinical disease typical of ordinary food-poisoning, and though some cases were serious enough to be admitted to hospital, none could be described as being dangerously ill, except the one fatal case where death was attributed to primary cardiac disease. After a six-month interval no patient is known to have suffered from sequelae. From this review it seems probable that though *Salm. paratyphi C, Salm. cholerae-suis,* the kunzendorf variety, and *Salm. birkenhead* are very similar in their biochemical and serological reactions, yet the type of clinical disease produced in man may be very different.

Summary

1. A new *Salmonella* type, *Salm. birkenhead,* with the antigenic structure VI, VII ; c ⟷ 1,6 has been described. The Vi antigen was not detected in any of the strains.

2. This organism was isolated from the faeces of eight cases of food-poisoning and one symptomless carrier.

3. The difference in antigenic structure and clinical disease caused by *Salm. birkenhead* and the closely related organisms *Salm. paratyphi C, Salm. cholerae-suis* and *Salm. cholerae-suis* var. *kunzendorf,* are discussed.

We wish to thank the many bacteriologists for sending cultures for identification, and Dr. A. M. McFarlan, who so kindly carried out the epidemiological investigation in the eastern region.

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PENICILLIN BLOOD LEVELS AFTER INJECTION OF SOLID AND SEMI-FLUID OILY SUSPENSIONS OF PENICILLIN

BY

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The object of injecting an oily suspension instead of an aqueous solution of penicillin is to secure delayed absorption from the depot in the muscle and to achieve a prolongation of effective penicillin blood levels from a single injection. Many vehicles have been recommended, but so far a suspension of penicillin in beeswax and arachis oil, as used by Romansky and Rittman (1944), has proved the most satisfactory. In a previous communication (J. Ungar, 1946), I have shown that the degree of prolongation of penicillin action depends, apart from individual variations in the absorptive capacity of experimental animals or patients, to a large extent on the consistency of the material (which is determined by the viscosity of the oil and by the amount of beeswax) and on the amount of penicillin in the suspension.

In a number of experiments performed since then we have repeatedly confirmed that the delayed absorption of penicillin is governed by the consistency of the final preparation; see, for example, Table I.

We have tried arachis oil, " alcoholized " arachis oil,* ethyl oleate, and cocoa butter and found that arachis oil is the most suitable medium for the suspension. We tried altering the concentration of beeswax from the original recommended by Romansky. We used suspensions of penicillin in oil containing 1, 2, 3, 4, and 6 per cent of beeswax, and we found that the amount of beeswax present in the final preparation is an essential factor in determining the absorption of penicillin (Tables I and II). Lowering the amount of beeswax to between 1 and 2 per cent results in a more fluid product with the obvious advantage of easier manipulation, but the blood levels after injection of these products are less satisfactory.

The technique of injecting the different preparations was constant, and possible variations of volume and temperature of the injected suspensions were rigorously controlled in all experiments.

* A mixture of ethyl esters of the total fatty acids of arachis oil.

The penicillin-oil-beeswax (P.O.B.) prepared according to the original method of Romansky has disadvantages from the point of view of administration, for it is solid at normal temperatures and has to be heated to about 50° C. before it can be injected. Anyone who has to administer the preparation knows how cumbersome it is to fill the syringe with the heated P.O.B. and inject it quickly before it solidifies. For this reason methods have been suggested to increase the fluidity of the preparation, not by decreasing the content of beeswax, but by one of the special means used for preparation of colloids.

Milled Penicillin and Beeswax

A product of this kind is a suspension of penicillin in oil and beeswax made by passing the mixture through a special colloid mill. This milled preparation, containing 4.5 per cent of beeswax, is semi-solid at room temperature; when heated to body temperature it can be easily drawn into a syringe and injected. Table I and the Figure show blood levels in rabbits injected with the solid

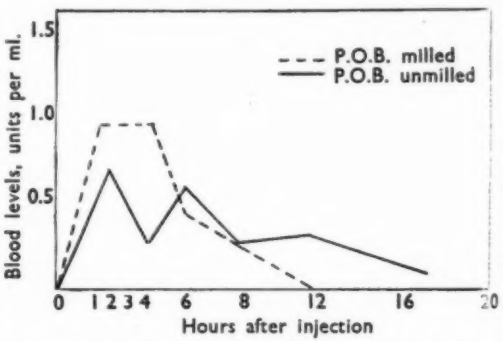

FIG.—Comparison of penicillin-oil-beeswax, milled and usual unmilled at room temperature (142,000 units per ml.) (20,000 units injected in 900 g. rabbits).

TABLE I

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 50,000 UNITS OF PENICILLIN IN OIL-BEESWAX UNMILLED SUSPENSIONS

Penicillin	% Beeswax	Rabbit no.	Hours after injection									
			2	4	6	8	10	12	14	16	18	20
Crystalline sodium	Nil	1	2.0	0	0	0	0	0	0	0	0	0
		2	2.0	0	0	0	0	0	0	0	0	0
	1	3	2.0	0	0	0	0	0	0	0	0	0
		4	2.0	0.03	0	0	0	0	0	0	0	0
	2	5	2.0	2.0	1.0	0.5	0.3	0.6	0	0	0	0
		6	2.0	2.0	1.0	0.4	0.1	0	0	0	0	0
	4	7	1.5	2.0	2.0	1.0	0.25	0.06	0.12	0.1	0.03	0
		8	2.0	2.0	1.0	0.5	0.3	0.12	0.12	0.03	0	0
Amorphous calcium	Nil	9	—	—	—	—	0	0	0	0	0	—
		10	—	—	—	—	0	0	0	0	0	—
	1	11	—	—	—	—	0	0	0	0	0	—
		12	—	—	—	—	0	0	0	0	0	—
	2	13	—	—	—	—	0.03	0.03	0	0	0	—
		14	—	—	—	—	0.12	0.03	0	0	0	—
	4	15	—	—	—	—	0.25	0.25	0.03	0	0	—
		16	—	—	—	—	0.25	0.1	0.1	0.06	0	—

and semi-solid preparations, both containing the same amount and type of penicillin and 4.5 per cent of beeswax.

We see from Table III that with this preparation we sacrifice part of the prolonged activity. Instead of a period of from sixteen to twenty hours during which penicillin is circulating in the blood after an injection of solid P.O.B., as reported previously (Ungar, 1946), we get, after the injection of a milled suspension, detectable blood levels up to a period of twelve to fourteen hours, which is satisfactory when treatment is given twice in twenty-four hours. In Table IV are given the blood levels of patients injected intramuscularly with 1 ml. of a milled suspension of penicillin containing 300,000 units, from which it can be seen that a level of up to 0.1 unit per ml. is maintained during a period up to six hours and a level of 0.06 unit per ml. up to eleven hours.

Discussion

Penicillin suspensions in oil and beeswax prepared by simple mixing of the components are at ordinary temperatures solid and difficult to

TABLE II

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 30,000 UNITS IN 0.1 ML. OF UNMILLED SUSPENSION

Sample	Penicillin	% Beeswax	Rabbit no.	Hours after injection						
				1	3	5	7	8	12	24
EPC 10/54	Crystalline sodium	1	17	4.0	0.03	0	0	0	—	—
			18	4.0	0.06	0	0	0	—	—
EPC 10/55		4	19	4.0	1.0	0.5	0.2	0.06	0	0
			20	4.0	1.0	0.25	0.03	0.03	0	0
EPC 10/56	Amorphous calcium	1	21	4.0	0.5	0.1	0.03	0	0	0
			22	4.0	0.12	0.03	0	0	0	0
EPC 10/57		4	23	4.0	1.5	0.75	0.5	0.4	0.06	0
			24	4.0	1.5	0.75	0.25	0.1	0	0

TABLE III

BLOOD LEVELS IN RABBITS AFTER INTRAMUSCULAR INJECTION OF 20,000 UNITS OF PENICILLIN-OIL-BEESWAX (MILLED AND UNMILLED)

Sample	Rabbit no.	Hours after injection						
		1	2	4	6	8	12	24
PC 1/16 (milled) ..	25	0.25	1.0	2.0	0.5	0.5	0.03	Nil
	26	0.5	1.5	0.5	0.5	0.25	Nil	Nil
	27	0.25	1.0	1.0	0.25	0.125	Nil	Nil
	Mean	0.33	1.16	1.16	0.42	0.3	0.01	Nil
PC 2/31 (unmilled)	28	0.5	0.5	0.25	0.7	0.25	0.125	Nil
	29	0.125	0.5	0.125	0.5	0.25	0.5	0.03
	30	0.125	1.0	0.5	0.5	0.4	0.5	Nil
	Mean	0.25	0.67	0.3	0.57	0.3	0.375	0.01

administer. It is possible to achieve fluidity at body temperature either by reducing the amount of beeswax from the original 4.5 per cent to 1 per cent or by submitting the suspension to a special milling process. The first alternative results in a marked drop in the time of circulation of penicillin in the blood ; the second method results in a partial reduction of the circulation time. For the milled preparation it is still essential to add 4.5 per cent of beeswax to the oil suspension ; otherwise the period of penicillin circulation in the blood drops. In our experiments we have observed that suspensions containing crystalline sodium penicillin or amorphous calcium penicillin give similar blood levels. Further, we have so far been unable to detect any effect of particle size of amorphous calcium penicillin, when administered as P.O.B., on blood levels, and a slight improvement in one experiment with crystalline sodium penicillin is of doubtful significance.

In using the milled penicillin beeswax suspension we make a compromise—to achieve an increased fluidity of the product we sacrifice to some extent the prolonged period of blood levels following injection of a solid penicillin suspension. The results of our tests confirm our earlier finding of an inverse relation between the fluidity of penicillin suspensions and the time during which the penicillin circulates in the blood.

One additional point should be emphasized when comparing the blood levels of penicillin after injections of solid and milled P.O.B. The solid suspension usually gives blood levels up to a period of about eighteen hours, whereas the milled P.O.B. maintains blood levels over a shorter period, though the initial levels in the first one to three hours are higher than after injection of the solid P.O.B. This initial higher blood level (Table III and Figure) compensates for the reduced penicillin circulation in the blood and makes the milled suspension second only in value to aqueous solutions when higher bactericidal blood levels are required—blood levels essential to suppress micro-organisms less susceptible to penicillin, such as occasionally occur in cases of septicaemia or chronic localized infections.

Summary

1. The effect of penicillin in oil-beeswax suspension depends on the consistency of the material

TABLE IV

BLOOD LEVELS IN PATIENTS INJECTED WITH 1 ML. OF MILLED PENICILLIN SUSPENSION 300,000 UNITS PER ML.

Patient	Hours after injection										
	1	2	3	4	5	6	11	15	16	18	20
1	0.25	0.25	0.25	0.125	0.125	0.1	0.06	0.06	0.06	0.03	Nil
2	2	1	1	0.5	0.5	—	0.06	} Not tested further			
3	2	2	1	1	1.5	0.2	—				

and the amount of penicillin present. Amorphous calcium penicillin and crystalline sodium penicillin give equally effective products.

2. The amount of beeswax is one of the essential factors in delaying the absorption of penicillin.

3. The "milled" penicillin beeswax suspension is characterized by its ease of administration and gives blood levels midway between those following injections of the solid penicillin-oil-beeswax or of aqueous solutions of penicillin.

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Addendum

At the time of reading the proof of this communication, which had been reported at the January, 1948, meeting of the Association of Clinical Pathologists, I came across a paper by Dowling and others on "liquid" versus "solid" penicillin in oil and wax (*J. Amer. med. Ass.*, **135**, 567). This report gave the results of treatment of patients with fluid beeswax suspension. Their investigation seems to indicate that fluid suspensions may have similar absorbtion-delaying effect as solid. suspensions if the former are prepared from penicillin particles of 50 μ size or larger.

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EMBOLISM AFTER PENICILLIN-OIL-BEESWAX

BY

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The occurrence of fat embolism after injury to bone has been described and the literature adequately reviewed by Robb-Smith (1941) and by Rowlands and Wakeley (1941). Similar findings occur after the accidental intravenous introduction of oily substances. Grossmann (1946) described the *x*-ray appearances of two cases of pulmonary oil embolism following salpingography. The first patient had no symptoms for two days ; the second patient began to cough very soon after the injection of the oil. The *x*-ray films in the latter patient were taken within five minutes, when it was seen that the main filling of the pulmonary arterial tree occurred in the lower lobes. Many observers have stressed the delayed appearance of symptoms which occur after fat embolism. Harris and others (1939) suggested that this is due to hydrolysis of fat particles with the release of free fatty acids. Animal experiments that were carried out suggested that severe reactions may occur after an oil-beeswax mixture, not only because the mixture blocks large branches of the pulmonary artery, but also because of the severe inflammatory reaction elicited by beeswax.

When a patient dies the recognition of a fat or oil embolism is not easy, because special methods have to be used to show the presence of these substances in the sections. In patients who recover the clinical picture is not clearly defined or easily recognized because the diagnosis has not been considered, especially where the symptoms are delayed. The development of pulmonary and cerebral signs and symptoms with petechial haemorrhages in the skin should all be looked for. Fat globules in the sputum and/or urine confirm the diagnosis. In many cases no abnormal signs are present and the symptoms are minimal.

I can find only one previous published account of oil embolism following penicillin in oil-wax therapy. Bondy and others (1947) describe a patient who had a severe reaction which showed maximal radiological changes on the second and third day after injection. The immediate effects were slight, consisting only of a penicillin-like taste in the mouth and a few coughs.

Two cases that occurred over two years ago had symptoms corresponding to a third described below, in that these symptoms were immediate and minimal, consisting of a tight feeling in the chest and a cough immediately after receiving an injection of penicillin in oil-wax.

Case Report

A married woman, aged 30, had been admitted for streptomycin therapy for persisting ulcerative colitis. While in the ward she had complained of chronic bronchitis, and by the time she had finished the streptomycin therapy there was no doubt that she had developed true asthmatic attacks. While awaiting discharge from hospital she was given an injection of 600,000 units in 2 ml. of calcium penicillin. The penicillin was suspended in peanut oil with 4.8 per cent (w/v) beeswax *B.P.* The injection was given by a nurse who, on being questioned, could not be certain that the usual precautions of making sure that the needle was not in a vein had been carried out. Immediately after the injection the patient began to cough, said she felt sick, asked for a bed-pan, and then said she was going to faint. She was put back to bed and when seen five minutes later she said she was feeling quite well. She was surprised the injection had made her feel faint, as previously she had had many injections of either penicillin or streptomycin without ill effect. When she was examined there were no abnormal physical signs, other than those due to generalized bronchitis which had been noted previously. The bronchitis was productive of a little sputum, about 20 ml. of which was collected during the following day: this amount represented the expectoration from the time of waking until before the midday meal. Unfortunately no precautions were taken to make sure that this was not contaminated with any oil or fat from a food source. A further specimen of sputum was collected three days later and a control specimen of sputum taken from another

chronic bronchitic patient. Sudan III was used as an oil stain. Only in the specimen collected the first day after the penicillin injection and only in certain parts of the field could many oil globules be seen ; all these were extracellular, and most of them were very minute—about 5 μ in diameter—though a few of the large ones were 50 μ in size. Examination of a slide with the stained sputum on it, and a control of the unstained and also stained penicillin in oil-wax, showed that in no case was there rotation of the plane of polarized light. It is therefore suggested that the oil in the sputum had as its source the penicillin oil-wax injection given into the buttock.

Summary

A case is described in which a mild immediate reaction occurred after an injection of penicillin in oil-wax. It would appear that this was due to oil embolism.

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J. clin. Path. (1948), **1**, 246.

TECHNICAL METHODS

ANOTHER WAY OF APPLYING IMMERSION OIL

BY

J. STEVEN FAULDS

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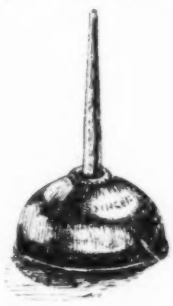
(RECEIVED FOR PUBLICATION, MARCH 19, 1948)

In a recent short paper published by Pijper (1947) an improved method of applying immersion oil to slides is described. It seems odd that laboratories should still employ the archaic method of applying the oil from a bottle by a rod, when not even a garage would dream of employing this method. I have tried many shapes of bottles and types of rods—made from glass, cane, or spring steel—and all of them were equally messy, wasteful, irritating, and inefficient; if the bottle is too full a drop falls on the bench before the microscope is reached, and if the bottle is nearly empty insufficient oil adheres to the rod and two or three applications are necessary before the correct amount is obtained, and usually air bubbles are included. After reading Pijper's article I wondered if anyone had ever tried an oil-can.

The ideal oil-can for immersion oil must be flat-bottomed so as to stand upright on the bench, and must have a long, tapering stem and a narrow opening—all features being present in the original Singer oil-can.* An alternative oil-can called the Alton Valve Spout is nearly as good and is obtainable at any wholesale motor supplies distributors at a cost of 2s. 6d.; this has an additional advantage of a screw valve on the stem which can be closed by half a turn, and is useful when the oil-can is being carried in a microscope case, as it does not leak when lying on its side; a valve is unnecessary, however, when the oil-can is standing on the bench. The amount of oil being applied is kept completely under control by pressing the bottom, and if this is done carefully there is no excess to run down the side of the stem and so soil the microscopist's fingers, the microscope, and the bench, wasting the immersion oil.



* Shortly available again from the Singer Sewing Machine Co., of Singer, Clydebank, Dumbartonshire, or agents, for 5d.

REFERENCE

Pijper, A. (1947). *J. Path. Bact.*, **59**, 486.

J. clin. Path. (1948), **1**, 247.

AN EMERGENCY BOTTLE FOR DRIED PLASMA

BY

WALTER KOCH

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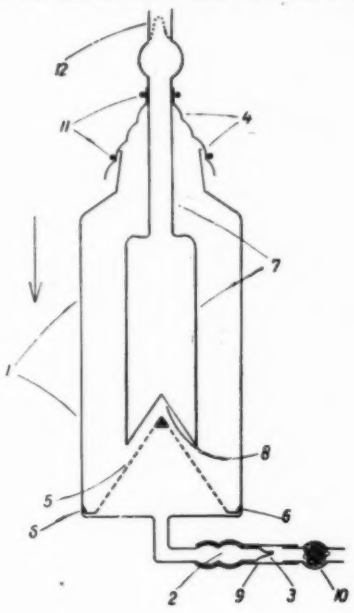
(RECEIVED FOR PUBLICATION, APRIL 24, 1948)

Dried plasma is usually kept in evacuated, rubber-stoppered bottles. Reconstitution calls for a series of additional gadgets as the water has to be transferred from its bottle to the plasma bottle, and two rubber tubings have to be inserted in order to give access to the air and to carry the dissolved plasma. Furthermore, if the vacuum in the plasma bottle has decayed, the water has to be pumped over with a blowing ball. All these manipulations are not always easily performed in the field.

An attempt has therefore been made to design an emergency bottle for quickly reconstituting dried plasma. The underlying principle is that of the fire-extinguisher in which the contents are mixed after an ampoule has been crushed.

The Emergency Bottle

The emergency bottle (see Figure) consists of a cylindrically shaped outer vessel (1) whose lower end forms an olive (2), drawn out to a point and sealed (3). The wide upper mouth of this vessel forms a brim which engages a bellow-shaped rubber-connexion (4). On the bottom of the vessel rests a cone of stainless steel (5) with reinforced tip. This cone is a sieve with holes of about 1½ mm. diameter resembling an inverted filter-cone. Its rim is flattened and is kept towards the bottom of the outer vessel (1) by four protrusions of the glass (6). The inner vessel (7) is a big ampoule with a stout neck which passes the rubber-connexion (4). The bottom (8) of the ampoule is drawn in and thinned. The neck of the ampoule is dilated near its top after the rubber-connexion (4) has been pushed over. This bellow-shaped rubber-connexion is cheaply available as rubber push-on connector for gas or water tubing; for larger bottles small rubber showers that may be attached directly to taps are a satisfactory alternative if the perforated plate is removed. A short piece of thick (pressure) rubber tubing (9), pushed over the olive, protects the drawn-out tip (3), and leads to a glass bulb (10). This bulb (10) is stuffed with 28- to 32-gauge bandage and acts as a filter. The free end of the bulb is connected to a needle protected against contamination (not shown in the drawing). A sintered glass pipeline filter would probably be better than gauze bandage, but this could not be tried*; when assembling the apparatus, distilled water is filled into the outer vessel (1) and all parts are sterilized in the autoclave. The connector (4) is then secured to both vessels by circular wires (11). The distance between the bottom of the ampoule (8) and the steel cone (5) is determined by the flexibility of the rubber piece (4) so that accidental movements of the ampoule should not engage the steel cone. The ampoule (7) is dried by connecting to a pump, and dried plasma is then filled into the ampoule. The bulb in the neck of the ampoule is plugged with sterile cotton wool, evacuated, and sealed at (12) (dotted line).



When needed, the inner vessel (7) is pushed into the outer (1), in the direction indicated by the arrow. As soon as the bottom of the ampoule (8) is crushed, water is drawn in and mixes with the dry plasma. Shaking aids dissolution of the plasma. Then the tip of the ampoule (12) is opened to give access to the air, and flow of the plasma is started by bending the rubber tubing (9) whereby the tip (3) is being broken. The vessels can be re-used.

* Messrs. Baird and Tatlock, London, recommend their K.301 H.3 or K.301 H.4 filter tube as a suitable sintered filter.

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ESTIMATION OF STREPTOMYCIN AND PENICILLIN IN BLOOD

BY

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From the Bristol Royal Hospital (Infirmary Branch)

(RECEIVED FOR PUBLICATION, APRIL 14, 1948)

The method here described, which we have been using for some time in routine work, is a modification of the micro-method of May and others (1947), in which dilutions of the patient's serum are added to phenol-red-serum water on waxed slides, and incubated in capillary tubes.

The dilutions of patient's serum and their addition to the medium are performed on a waxed glass plate which is incubated directly, without filling the mixtures into capillary tubes and fixing to plasticine-covered slides. In this way the amount of manipulation is reduced and an appreciable saving of time is achieved, especially when several sera are being titrated at the same time. Moreover the colour change at the end point is even more obvious than in capillaries and there is no danger of upsetting the tests by rough handling.

Details of Method

The medium used is 1 per cent glucose in a mixture of serum 1 part, distilled water 4 parts, sterilized by steaming (May and others, 1947). We find it best to make up batches of this medium without indicator. A saturated solution of phenol red in distilled water is put up separately in small amounts, each sufficient for one batch of medium, and similarly sterilized. At the time of the test the indicator is added in the proportion of about 0.05 ml. to 1 ml. serum water. We have found that if the indicator is added to the medium some days before use, fading occurs and the colour changes after incubation are not so distinct. The medium is inoculated as in the original method (0.01 ml. of 24-hour broth culture of the test organism to 1 ml. medium).

The capillary pipettes used to measure unit volumes have tapered, slightly angled free ends; as in the original method, they are graduated to contain 0.025 ml.

For making and incubating the dilutions, waxed rectangular slabs of glass, in which are drilled a series of depressions, are used. The slabs are cut from plate glass, about 7 mm. thick, and measure approximately 7.5×4.5 cm. In one surface of each slab are drilled ten circular depressions 8 mm. in diameter and about 2 mm. deep at the centre. Each plate is placed in a 3½-in. Petri dish and sterilized in hot air. When cool it is grasped with sterile forceps and quickly dipped in melted sterile paraffin wax at about 60° C., briefly drained, and replaced in its dish.

Serial dilutions of the patient's serum and of a suitable standard solution of antibiotic in normal serum are made in equal volumes of sterile saline in the depressions (with the last as a control). To each is added an equal volume of inoculated medium. A quantity of water or of moistened filter paper is placed in the Petri dish, which is incubated, lid upwards, at 37° C. During incubation the dish should rest on wood or other heat-insulating material to avoid moisture condensation on the lid.

For streptomycin estimations we use klebsiella 41 as test organism and inactivate the patient's serum. For penicillin, the Oxford staphylococcus is used. As in the original method, it is advisable to aim at having the end point within the first five or six dilutions in order to minimize the cumulative error of the wash.

After incubation, the last dilution showing complete inhibition is taken as the end point. The strength of antibiotic is calculated by comparison with the standard. The drops of fluid retain their convex surfaces in the waxed depressions, thus rendering colour differences more striking; there is no tendency for them to overflow, even with quite rough handling (Plate IX).

Numerous titrations have been done in parallel with the capillary tube method, without significant differences in results.

Recently we have found (Goslings, 1947) an account of an essentially similar method for penicillin, using haemolytic streptococcus, with blood as the indicator.

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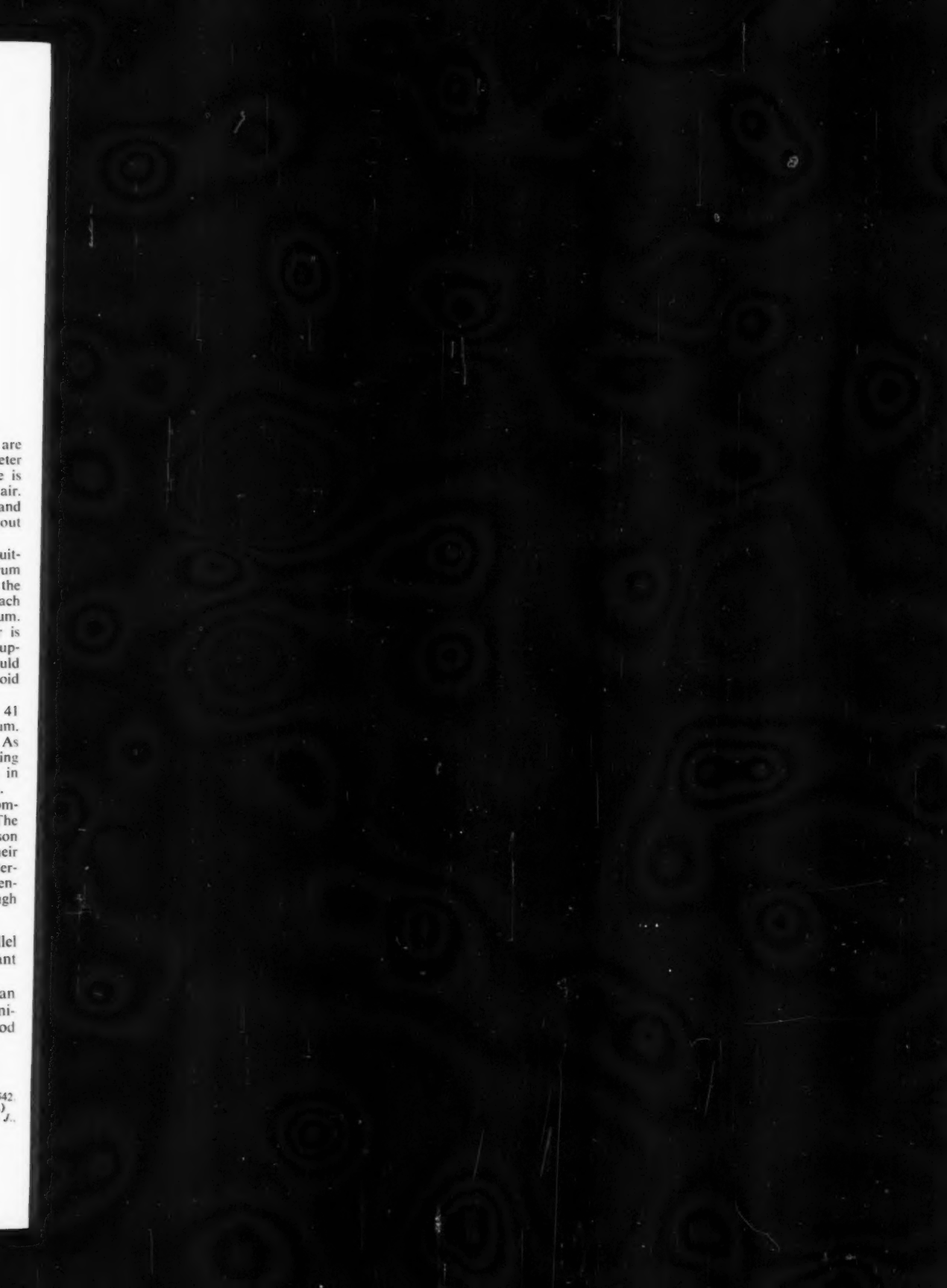

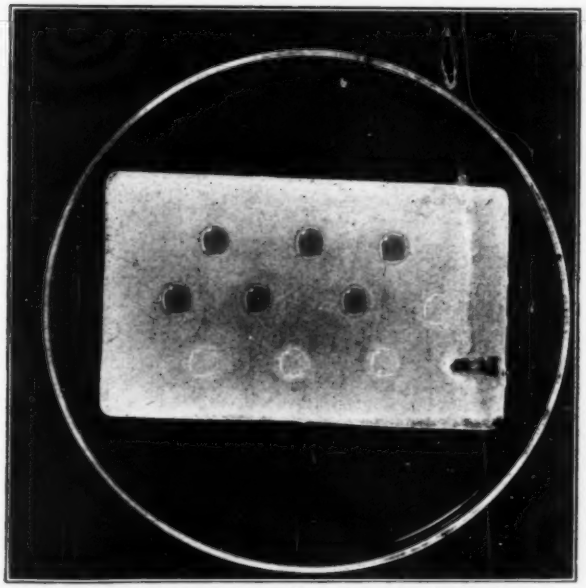

PLATE IX.—Waxed depression plate after incubation.
End point at dilution 1/64.

REVIEWS

Il Principio Antianemico-Pernicioso. By G. Astaldi and M. Baldini. Pavia: Il Farmaco, Monograph. January, 1948. Pp. 234, figs. 54. Price 1,200 lire.

This monograph on the pernicious anaemia factor by two members of Ferrata's school of haematology appears at a moment when the discovery of folic acid and the production of new and powerful liver concentrates have created fresh interest in and raised new questions about the origin and treatment of the macrocytic anaemias. The authors have set out to give an interim review of this rapidly changing subject, and have produced a clear and up-to-date summary of its theoretical and practical aspects.

The book is divided into two parts: the first deals with the nature of the liver principle and of the extrinsic and intrinsic factors, the properties of the various commercial and experimental liver extracts, desiccated stomach preparations, and the pteroylglutamic acid group. In a very full review of the older preparations covering both the English and continental literature, the only important omission is that of crude " proteolysed " liver. Recent work on folic acid, its conjugates, and thyamine is adequately described. The second part covers the methods of assaying the activity of therapeutic preparations both from the theoretical and practical aspects, and includes much of the author's original work. After a critical account of the common therapeutic tests, based on the reticulocyte and erythrocyte responses, they present a review of many other suggested methods of assay, all of which they reject as either non-specific or as yet insufficiently controlled. There are in fact no rapid or large-scale assay methods, and this explains most of the difficulties in research on new preparations. The author's own solution of the problem is based on studies of bone marrow both *in vivo* and *in vitro* and consists in a statistical analysis of the diminution in size, under treatment, of the red blood cell precursors; but, as it involves multiple marrow punctures and a laborious technique, it is doubtful if this attractive suggestion will be widely adopted in practice.

Defects in an otherwise excellent book are the careless quotation of references and the absence of an index. In spite of these, it can be recommended to anyone in search of a full and balanced account of present-day knowledge of the nature and application of the substances used in the treatment of pernicious anaemia.

F. W. GUNZ.

Major Endocrine Disorders. By S. Leonard Simpson. Geoffrey Cumberlege, Oxford University Press. Second edition, 1948. Pp. 552+xxii. Price 42s.

The new edition of this book gives a good picture of the recent development of endocrinology. This speciality is growing fast, and it will be increasingly difficult for the general physician to keep abreast of its progress. The way the book is written will help him in this task. Each endocrine organ is treated separately; after a short review of its physiology the clinical disorders connected with its dysfunction are dealt with. These are often accompanied by typical case histories and by excellent photographs. The pituitary gland and the gonads are given more space than the other endocrine organs; in the latter some disorders are treated which cannot be strictly termed major disorders, but this is probably unavoidable. The chapter on the pancreas and diabetes mellitus is comparatively short. Biochemical methods used in the diagnosis of the disorders described are not given, and the biochemical data are sometimes scanty, but this is compensated by the introduction of ample references. On the whole, the author has successfully avoided cramming his book with too much detail; it will be a welcome help to the student and to the clinician.

H. HERXHEIMER.

Nutrition and Hormones. By L. T. Samuels. American Lecture Series No. 11. Blackwell Scientific Publications, Oxford. 1948. Pp. 48. Price 8s. 6d.

This is a series of short essays on the mutual interaction of hormones and nutritional factors. It aims at correlating the gross metabolic changes in endocrine diseases on the one hand with the effect of various types of malnutrition on the endocrine glands on the other. Since these materials make up the greater part of modern endocrinology the result is a rather brief account of the relationships of the various hormones which is perhaps too incomplete to be of value to the average pathologist or clinician. For example, the effect of iodine deficiency on the thyroid gland is dismissed in six lines and that of thiouracil in four. The book is attractively bound and the well chosen bibliography of 108 recent references is a valuable introduction to the literature.

N. F. MACLAGAN.

OBITUARY

JAMES McINTOSH

On April 5 this year Pathology suffered a great loss in the death of James McIntosh, Professor of Pathology in the University of London and Director of the Bland-Sutton Institute of Pathology at the Middlesex Hospital since 1920. He was a man of wide interests, with an international reputation as an authority in several spheres of pathological research.

He was educated at Robert Gordon's College and at the University of Aberdeen, where he qualified in 1905 and was awarded the M.D. with highest honours in 1908. After spending two years with Levaditi at the Pasteur Institute during his tenure of the Alexander Anderson Scholarship, he returned to Aberdeen in 1908 as Carnegie Research Scholar. In 1910 he became a Grocers Research Scholar at the London Hospital, where he worked in association with William Bulloch, Paul Fildes, and H. M. Turnbull until 1920 when, at the age of 37, he was appointed Professor of Pathology and Director of the Bland-Sutton Institute.

His first outstanding contribution to medical science was his pioneer work with Fildes on the perfection of the Wassermann reaction and the application of Ehrlich's new remedy, salvarsan, in the treatment of syphilis. This investigation was a natural sequel to his researches with Levaditi at the Pasteur Institute on *Treponema pallidum* and other spirochaetes. It was there, also in collaboration with Levaditi, that he performed some of the earliest experiments on the development of resistance to arsenical drugs in micro-organisms. He was always interested in the problem of drug resistance, and he returned to it almost forty years later to make fundamental contributions to our knowledge of the development of resistance in bacteria to the sulphonamides, penicillin, and the acridines. His exceptional grasp of the fundamentals of chemotherapy was again shown in the views he expressed with Whitby in 1939 on the mechanism of sulphonamide action, which were later confirmed and brought to fruition by the discovery of the role of *p*-aminobenzoic acid by Fildes and Woods, who were then housed in his Institute. He also played an important part in the clinical application of the new antibacterial drugs, particularly with respect to the bacteriological control and methods of administration—as will be recalled by members of the Association of Clinical Pathologists who heard his address on subacute bacterial endocarditis at the winter meeting two years ago.

JAMES McINTOSH

An achievement which alone would have established his reputation in medical research was his classification of the anaerobic bacteria associated with war wounds. He took up this subject during the 1914–18 war, and it is not too much to say that his work made order out of the chaos then existing because of the inadequacy of the methods in current use for the isolation of these organisms. In this difficult subject he displayed great technical dexterity in the new methods he introduced and, with Fildes, he evolved the anaerobic jar that bears their names and is the

indispensable apparatus for the critical study of anaerobes. His report to the Medical Research Council on anaerobic infections is a classic used by all workers in the most exacting discipline in all bacteriology. His services in this field were again called upon in the 1939–45 war, when he was appointed a member of the War Wounds Committee of the Medical Research Council, who profited not only from his wide experience but also from the investigations he conducted on many urgent problems. Indeed one would not hesitate to say that the high standard of the work done on anaerobic infections during the last war was largely due to his lifelong interest in the anaerobic bacteria and to the inspiration he gave to many other workers.

Another sphere in which McIntosh gained distinction was the experimental study of virus diseases. He was first actively involved in this field at the London Hospital where, with H. M. Turnbull, he commenced a study of encephalomyelitis following vaccination, which he continued at the Bland-Sutton Institute. In the report on this subject to the Ministry of Health in 1925 McIntosh found himself so much at variance with his colleagues that he submitted a minority report expressing his conviction that vaccinia virus could not be excluded as a cause of post-vaccinial encephalomyelitis. It was not until many years later that his views on the generalization of vaccinia virus and the causation of post-vaccinial encephalitis became widely accepted. In later years he applied his experience in handling viruses to the experimental study of cancer. He made the important observation that tumours induced by tar in fowls could be transmitted by cell-free filtrates. Although other workers have brought forward indirect evidence in support of his findings, none has succeeded in repeating his demonstration of a virus agent in these induced tumours. He was firmly of the opinion that viruses played the dominant part in the aetiology of tumours, and, with the further inroads that are being made by virologists into the tumour problem, his work is now gaining increasing respect. In his handling of viruses his mechanical genius came to the fore—as it had done before in the design of the anaerobic jar—as exemplified by his adaptation of the spinning-top ultracentrifuge for the sedimentation of viruses and his modification of the Sharples centrifuge for the continuous centrifugalization of large quantities of virus suspensions.

McIntosh will also be remembered for the smooth-running organization he built in the Bland-Sutton Institute, which he administered from 1920. Here, besides carrying on his many scientific investigations, he was responsible for undergraduate teaching in pathology and also for the pathological services to the Middlesex Hospital. His outside activities in the service of pathology were many. He was a member of the Pathological Society of Great Britain and Ireland, for which he acted as treasurer for many years, a senior member of the Medical Research Club, a past-president and representative to the Library Committee of the Pathological Section of the Royal Society of Medicine, and an honorary member of the Association of Clinical Pathologists. He also took a deep interest in the welfare of his laboratory technicians and did great service for many years in the Pathological and Bacteriological Laboratory Assistants Association, now the Institute of Medical Laboratory Technology. He was also an examiner to the Universities of London, Cambridge, and Manchester, and for the Conjoint Board.

In the 1939–45 war he was pathologist to Sector V of the Emergency Medical Service and directed two laboratories in the Aylesbury district in addition to the Bland-Sutton Institute. As Chairman of the London Sector Pathologists Committee he also played his part in co-ordinating the pathological services of the London area. In addition to his work in the Sector and for the Medical Research Council, he also conducted an investigation of cases of encephalomyelitis on behalf of the Ministry of Health.

McIntosh was a bachelor of a rather retiring disposition, and to strangers and even to many acquaintances he was sometimes difficult to understand. His shyness and habit of self-effacement frequently gave the impression that he was somewhat brusque and disinterested. Those who knew him well, however, were aware that it cost him a great effort to show his displeasure and that when he did so some fundamental principle was involved. Misunderstanding also sometimes arose from his way of expressing himself, which led to apparent contradictions, largely because of his habit of letting his thought outstrip his speech. Even so, one was frequently startled by the rapidity with which he got to the core of a complicated subject and summed it up in a few words by a process which defied analysis.

He had many interests outside his profession, such as his series of high-powered cars, his golf, and his garden. He was a genial host and helped many in their private worries. There are many who will miss McIntosh, not only as an inspiring chief but also as a loyal friend.

F. R. SELBIE.

We regret to record the death of Dr. Gordon Wilkinson Goodhart; an obituary notice will appear in the next issue.

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ABSTRACTS

This section of the JOURNAL *is published in collaboration with the two abstracting journals, Abstracts of World Medicine, and Abstracts of World Surgery, Obstetrics and Gynaecology, published by the British Medical Association. In this* JOURNAL *some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.*

BACTERIOLOGY

Improvement of the Diagnosis of Tuberculosis by Guinea-pig Inoculation. (Amélioration du bactério-diagnostic de la TBC par l'inoculation au cobaye.) BADOUX, V. (1947). *Schweiz. Z. Path. Bakt.*, **10**, 470.

A review is made of technical details of guinea-pig inoculation in the diagnosis of tuberculosis, and a plea is entered for always basing a report on results in 2 guinea-pigs. The material should be kept, and used to inoculate additional guinea-pigs should the originally inoculated animals die prematurely. The author claims an increase of 4.76% positive results since he has used 2 animals for every test. *R. Salm.*

Detection of Mycobacterium tuberculosis by Means of Fluorescence Microscopy. BRIGGS, G. O. A., and JENNISON, M. H. (1947). *Tubercle, Lond.*, **28**, 189.

The authors tested the value of the fluorescence method as used in a sanatorium of 210 beds admitting all types of pulmonary tuberculosis. A series of sputum smears was stained by the auramine-phenol technique (*Brit. J. Tuberc.*, 1946, **111**, 98). After examination these films were then re-stained by a Ziehl-Neelsen technique described by Jennison (*Bull. Inst. Med. Lab. Techn.*, 1946, **6**, 87). Of 500 smears thus examined 44.4% were positive with the fluorescence technique and 45.8% by the modified Ziehl-Neelsen method. Thus the modified Ziehl-Neelsen method yielded 1.4% more positive results with an expenditure of 6 seconds less time per smear, or an overall saving of 50 minutes.

Detection of Latent Genital Tuberculosis by Culture of Menstrual Discharge. HALBRECHT, I. (1947). *Lancet*, **2**, 947.

Endometrial biopsies have shown that latent genital tuberculosis is commoner than hitherto suspected, occurring in at least 5% of cases of primary sterility. Endometrial biopsy, however, can give a positive result only when tuberculosis has reached that particular portion of the endometrium removed for scrutiny. The author undertook culture of menstrual blood obtained with a vaginal speculum from the fornix and the os on the first or second day of the menses, and repeated his examination at least thrice in each patient. Of 140 tests in 80 women with primary sterility, including 10 with proved endometrial tuberculosis and 2 others clinically suspected of genital tuberculosis, culture was positive nine times in 6 women. The author suggests that this is the only safe method of establishing the diagnosis where genital tuberculosis is clinically suspected.

Culture of menstrual blood was positive in only 2 of the 10 proved cases of endometrial tuberculosis.
J. A. Chalmers.

Alcohol as a Disinfectant Against the Tubercle Bacillus. SMITH, C. R. (1947). *Publ. Hlth Rep., Wash.*, **62**, 1285.

The author reviews previous work on the efficiency of alcohol as a disinfectant, particularly against the tubercle bacillus. Water is essential to the disinfectant action of ethyl alcohol; the optimum strength appears to be 50 to 70%. The germicidal effectiveness of aliphatic alcohols increases with molecular weight as far as the amyl derivatives, and then decreases through octyl to undecyl alcohol. Normal propyl alcohol is the most effective.

Experimental data are given, and it is concluded that both ethyl and *iso*propyl alcohols are useful and practicable disinfectants against the tubercle bacillus and that they are specially suitable for skin disinfection. They are also suitable for thermometers and for surfaces, dishes, and handicraft articles which might be damaged by other methods of disinfection. Some plastics, painted and varnished surfaces, and some fabrics and dyes, may be damaged by alcohol, and in thoracoscopes and cystoscopes the lens systems may be held in position by alcohol-soluble cements and so may be liable to harm.
R. J. Lumsden.

Relative Numbers of Resistant Tubercle Bacilli in Sputa of Patients before and during Treatment with Streptomycin. PYLE, M. M. (1947). *Proc. Mayo Clin.*, **22**, 465.

In 7 out of 8 cases of pulmonary tuberculosis a few moderately resistant organisms were present in cultures from sputum before the start of chemotherapy. During treatment with streptomycin the proportion of resistant bacilli in cultures recovered from sputa at weekly intervals increased, the degree of their resistance increased, and the predominantly sensitive reactions of the isolated strains, as judged by the ordinary methods of testing sensitivity, were replaced by predominantly resistant reactions. *P. D'Arcy Hart.*

Estimation of Streptomycin in the Blood and Cerebrospinal Fluid. (Titrage de la streptomycine dans le sang et le liquide céphalo-rachidien.) CHABBERT, Y., and SUREAU, B. (1947). *Ann. Inst. Pasteur*, **73**, 1142.

The test organism used is the Oxford strain of *Staphylococcus aureus* grown in glucose broth with phenolsulphonphthalein at *p*H 8.4 as indicator. The organism

is inhibited in this medium by 0.44 unit of streptomycin per ml. A series of control tubes containing various dilutions of streptomycin is read against dilutions of the fluid, serum, plasma, or cerebrospinal fluid in the medium, the presence of growth being shown by change in the indicator. The tubes showing complete inhibition and just failing to show inhibition are noted. The method is sensitive to 0.44 unit per ml., and can be applied to urine provided the urine is sterilized by passage through a Seitz filter. *G. M. Findlay.*

Further Observations on the Occurrence of Streptococci of Groups other than A in Human Infection. FOLEY, G. E. (1947). *New Engl. J. Med.*, **237**, 809.

The grouping of strains of streptococci isolated from sources other than the upper respiratory tract was studied. Out of 118 cases investigated, 95 showed organisms of strains other than group A: 77 belonged to groups B, C and G, D, E, F, and K. The streptococci most frequently encountered in the study were those of group D, and these were usually associated with endocarditis, urinary tract infections, and intra-abdominal abscesses. Streptococci belonging to groups B, C and G, E, F, and K were met with in adult and childhood infections. The author believes that the incidence of streptococci of groups other than A cannot be properly ascertained if only colonies giving a beta reaction on blood agar are studied. *J. Smith.*

Staphylococcal Infection Due to Penicillin-resistant Strains. BARBER, M. (1947). *Brit. med. J.*, **2**, 863.

In a series of examinations undertaken during April–November, 1946, 99 patients yielded 14 strains of *Staph. pyogenes* from infected lesions resistant to penicillin; during February-June, 1947, 38 out of 100 patients yielded resistant strains. It would appear that 10 from the last group also harboured sensitive strains at the beginning of treatment. The sensitive strains were found to belong to serological and bacteriophage types different from those of the resistant strains.

The author brings forward evidence to show that the penicillin-insensitivity is not due to resistance built up in the tissues after administration of penicillin, but that the strains are originally resistant to the action of penicillin. They are penicillinase producers and flourish in a lesion only after the sensitive strain has been overcome by penicillin.

In determining the penicillin-sensitivity of these penicillinase-producing organisms the size of the inoculum was found to be important. The ditch-plate method was particularly valuable, for unless the size of the inoculum was carefully controlled some of the other methods for the determination of sensitivity might yield misleading results with penicillinase-producing bacteria. *H. J. Bensted.*

Treatment of Diphtheria Carriers with Penicillin. (La profilassi penicillinica nei portatori di bacilli difterici.) TORRICELLI, C., and MONTAGNA, A. (1947). *Lattante*, **18**, 474.

A series of 414 children, mostly infants, was examined for diphtheria organisms. Of the 22 strains recovered, 1 was a toxic *Corynebacterium diphtheriae*, 12 were non-toxic *C. diphtheriae*, and 9 were "pseudo-diphtheria" bacilli. The gravis organism was isolated from a child who had a sero-sanguineous nasal discharge and was probably suffering from diphtheritic rhinitis. The other organisms came from the nasopharynx of healthy carriers and from pus in 3 cases of chronic purulent otitis media. All 22 children were treated by the nasal or aural instillation of penicillin solution, 1,000 units per ml., 4 to 6 times a day for about 10 days, and the organisms disappeared. *E. G. Sita-Lumsden.*

The Suppressive and Schizonticidal Value of Paludrine (100 mg.) in Vivax Malaria. WOODRUFF, A. W. (1947). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 263.

Fifteen patients suffering from benign tertian malaria were treated with a single 100-mg. tablet of "paludrine." The results compared unfavourably with those obtained in a similar series treated by quinine for 3 days followed by mepacrine. It is suggested that this dose of paludrine is sub-optimal in an acute attack. Twenty patients, after an acute attack of benign tertian malaria treated by paludrine, were given 100 mg. paludrine weekly for 6 months. No toxic manifestations were noted. During the period of paludrine administration one short-term relapse and three possible clinical attacks occurred. During the 6 months following the period of paludrine administration there was 1 proved long-term and 1 possible clinical relapse. Thus, in the dosage used, it seems that paludrine does not invariably prevent long-term relapses. *J. L. Markson.*

Contribution to the Serological Diagnosis of Amoebiasis. (Contributo allo studio della sierodiagnosi nell' amebiasi.) RITA, G. (1947). *Riv. Parassit.*, **8**, 113.

The author carried out complement-fixation tests with an antigen made by Craig and Scott's technique on 63 patients with amoebic infection and on numerous controls: 14 had acute dysentery, 2 amoebic hepatitis, and 47 intestinal amoebiasis. In 1 patient with hepatitis the entamoebae were not found, but they were seen in all the others. Fifty-eight gave a positive result. Of the 5 negative cases, 2 had acute dysentery and 3 had mild intestinal forms. Persistence of the complement-fixation reaction indicates that the patient is not free from his amoebae and relapse is likely.

Meningitis Leptospirosa. BUZZARD, E. M., and WYLIE, J. A. H. (1947). *Lancet*, **2**, 417.

This paper records 5 cases aged 9 to 23 years in which Weil's disease was characterized solely by mild benign meningitis with certain particular features, especially suffusion of conjunctivae. Three had recently been river bathing and the other two had occupational risks. The cerebrospinal fluid had a slightly raised pressure and a cell count of 50 to 300 per c.mm. with varying proportions of granulocytes and lymphocytes. There was slight proteinuria, but no cells or casts in the urine. No patient was jaundiced. In all cases the agglutination titre rose to 1 in 1,000 or higher at the end of the second week. All recovered completely without specific treatment of any kind.

Meningitis due to Candida (Monilia) Albicans with Recovery. ZIMMERMAN, S. L., FRUTCHEY, L., and GIBBS, J. H. (1947). *J. Amer. med. Ass.*, **135**, 145.

This is believed to be the first recorded case of recovery from meningitis proved to be due to *Candida albicans*, the 3 previously recorded cases having all ended fatally. A man aged 28 had a week's history of increasing malaise, headache, fever, vomiting, and drowsiness alternating with delirium. White patches were present on the palate, posterior pharynx, and back of the tongue. He had neck rigidity, a positive Kernig sign, absent deep reflexes, and retention of urine. His cerebrospinal fluid contained 37 lymphocytes per c.mm. and 186 mg.

protein and 585 mg. chlorides per 100 ml. A direct smear of the fluid stained with methylene blue showed large, oval, budding yeast-like fungi, which could be cultured and subcultured with production of mycelia. Serum agglutination against *C. albicans* was positive at a 1 in 40 dilution.

The condition responded to an 8-day course of streptomycin, and he eventually made a complete recovery. *A. H. Stewart-Wallace.*

Observations on the Epidemiology of Poliomyelitis in Glasgow. ANDERSON, T. (1947). *Glasg. med. J.*, **28**, 328.

The epidemiology of poliomyelitis is discussed in the light of recent experience of the disease in Glasgow. It seems probable that the final number of cases in Glasgow in 1947 will not be far short of three-quarters of the total number of cases notified in the past 27 years. This high prevalence justifies a discussion of the possibilities: (*a*) that a new virus may have appeared; (*b*) that the virus normally present endemically may have acquired an increased power of attack; or (*c*) that the human herd may have become more susceptible because of some unknown environmental change. The author inclines to the view that person-to-person infection, chiefly from healthy carriers and abortive cases, is the most important means of spread. *W. H. Bradley.*

The Isolation of a Filterable Agent Pathogenic for Mice from a Case of Reiter's Disease. DUNHAM, J., ROCK, J., and BELT, E. (1947). *J. Urol.*, 58, 212.

Conjunctivitis was produced in mice by intraperitoneal injections of filtered allantoic fluid previously infected with material from the urethra and conjunctiva of a 23-year-old white man suffering from Reiter's disease with conjunctivitis, urethritis, and arthritis uninfluenced by sulphathiazole, penicillin, or neoarsphenamine.

The Harris Slide Test. A Microflocculation Test for Syphilis with Cardiolipin Antigen. VOGELSANG, T. M. (1947). *Brit. J. vener. Dis.*, **23**, 109.

Cardiolipin was used for the Venereal Disease Research Laboratory slide test by Harris *et al.*, who reported highly satisfactory results with a low incidence of false positive reactions (*J. vener. Dis. Inform.*, **27**, 169), as did Kline (*Arch. Derm. Syph., Chicago*, 1947, **55**, 514) when contrasting this test with the other flocculation procedures.

The present author employed what he christens the "Harris test" on 5,556 sera, and compared the results with the standard Kahn and Meinicke and with Bordet-Wassermann tests. Of 777 known syphilitic sera there was agreement between results of the Harris and Bordet-Wassermann in 81.85%. Of the remaining 141 sera 100 gave a positive Harris reaction and 99 a positive Wassermann, while negative results were obtained in 41 and 42 respectively. Of 4,779 non-syphilitic sera there was agreement in 98.72%, although, of the 61 remaining, 43 gave a negative Harris reaction and only 10 a negative Bordet-Wassermann. Thus the two tests were about equally sensitive, the Harris being the more specific. The Harris test showed similar sensitivity and somewhat greater specificity than the standard Kahn; even if less sensitive than the Meinicke, it was considerably more specific. *R. R. Willcox.*

BIOCHEMISTRY

A Survey of the Accuracy of Chemical Analyses in Clinical Laboratories. BELK, W. P., and SUNDERMAN, F. W. (1947). *Amer. J. clin. Path.*, **17**, 853.

In 1946 the Committee on Laboratories of the Medical Society of Pennsylvania carried out a survey to check the accuracy of findings in clinical laboratories. Some 59 pathologists in hospitals of all types expressed their willingness to analyse standard samples sent to them. Each received 12 samples, previously analysed, of aqueous solutions of glucose, chloride, urea, and calcium in concentrations commonly found in the blood, together with a sample of serum for protein estimation, and samples of whole blood for haemoglobin determination. Their findings were entered on plain printed cards, which were returned unsigned. The data were arranged in frequency distributions, and histograms are reproduced. The referee selected reasonable limits of error permissible for each analysis, and grouped results as "satisfactory" and "unsatisfactory" according as they fell within or outside these limits. The authors found a surprising degree of unreliability; measurements were below any reasonable standard of accuracy. Unsatisfactory results outnumbered the satisfactory ones, and gross errors were not uncommon. Of 51 haemoglobin determinations, only 17 were satisfactory (limits 9.8 ± 0.3 g. per 100 ml.); of 52 glucose determinations (limits 60 ± 10 mg. per 100 ml.) 19 were unsatisfactory; and of 44 serum albumin determinations only 9 were satisfactory. Roughly one-third of the calcium estimations were satisfactory, and two-thirds of the sodium chloride estimations.

A questionary on these unsatisfactory results brought replies from 106 pathologists. The majority attributed the inaccuracy to poor training and shortage of technicians, while 64 thought that there was lack of understanding between pathologist and staff.

S. S. B. Gilder.

(This very important survey should be read and the figures and tables studied in the original. A similar survey carried out in this country would certainly reveal the poor correlation existing among laboratories, even when they are using similar methods.—Ed.)

Methionine Excretion. Effect of Diet and Methionine Ingestion in Normal Subjects. TIDWELL, H. C., SLESINSKI, F. A., and TREADWELL, C. R. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 482.

The daily urinary excretion of methionine in 80 male medical students on a normal control diet containing approximately 2 g. methionine per day, as determined by the method of Albanese (*Bull. Johns Hopk. Hosp.*, 1944, **75**, 175), averaged 318 ± 9 mg. (range 199 to 518 mg.) or about 4.5 mg. per kilo body weight. The methionine excretion during 6-hour and 24-hour periods after the ingestion of 1 or 1.5 g. methionine was determined in 26 subjects given food and 11 subjects given none. The increase in methionine excretion was approximately 9 and 15% of the amino-acid supplement.

Three groups of 12 subjects were then placed on a diet with a low and a higher protein content and a high fat content respectively. The calculated daily methionine content of the diets was 1.34, 3.33, and 2.23 g. After 4 days no change in the methionine excretion was observed in the subjects on the diets with low or higher protein content, but there was a decrease in the methionine excretion in the subjects on a high fat diet, and a similar decreased excretion was observed when the latter

subjects were given a 1.5 g. supplement of methionine. Nine subjects who fasted for 3 days showed a diminished urinary excretion of methionine. It is suggested that an increased lipotropic requirement might account for diminished excretion of methionine in subjects on a high fat diet or after a 3-day fast. *C. C. N. Vass.*

Plasma *dl*-Methionine Levels Following Intravenous Administration in Humans. HARPER, H. A., KINSELL, L. W., and BARTON, H. C. (1947). *Science*, **106**, 319.

After a 12-hour fast a blood sample was withdrawn for determination of plasma methionine, and 50 ml. of 3% solution of *dl*-methionine was then given intravenously to 11 subjects. Methionine determinations were then carried out on the plasma and urine at intervals during 3 hours. Following a sharp rise in plasma methionine 15 minutes after injection the concentra ion slowly fell, but in no case did it reach the pre-injection level within the 3 hours of observation. There were considerable individual variations in rate of disappearance and in urinary excretion. The excretion was most rapid during the first 15 to 30 minutes when the blood levels were highest, but the amount excreted at any time was quite low when compared with the quantity injected.

D. T. Barry.

Use of Methionine and Vitamin Supplements in Treatment of Hepatic Disease. Clinical and Laboratory Observations. CAYER, D. (1947). *Arch. intern. Med.*, **80**, 644.

The author divides 18 cases into three groups: (1) 4 of acute infective hepatitis (2) 6 of chronic hepatitis without ascites, and (3) 8 of chronic hepatitis with ascites and signs of collateral venous circulation. The patients were given a daily oral dose of 3 to 6 g. of methionine and therapeutic doses of the vitamin-B complex, in addition to a diet of 3,500 calories made up of 120 to 140 g. of protein, 130 to 150 g. of fat, and 350 to 400 g. of carbohydrate given in multiple small feeds. Those in hospital were given 500 to 1,000 ml. of 10% dextrose solution intravenously daily. In group 1 the course of the disease was not appreciably altered. In group 2 considerable improvement, as judged by clinical observation and by liver-function tests, was noted in all cases within 7 days. In group 3, 7 out of 8 patients showed some clinical improvement, and 3 were well and carrying on full-time work on an average 13 months after treatment. The author comments on the well-known poor prognosis in this disease and quotes for comparison larger series reported by others and treated on different lines.

J. B. Mitchell.

The Thymol Turbidity Test as a Measure of Liver Disease. With Special Reference to Comparison of the Turbidity at 18 Hours with that at 30 Minutes ("18 Hour Turbidity Ratio"). SHAY, H., BERK, J. E., and SIPLET, H. (1947). *Gastroenterology*, **9**, 641.

The Thymol Turbidity Test and Impaired Liver Function. MANN, F. D., SNELL, A. M., and BUTT, H. R. (1947). *Gastroenterology*, **9**, 651.

Studies of Responses of Certain Hepatic Tests in Diseases of the Liver and Biliary Tract. I. Serum Cephalin Cholesterol Flocculation, Thymol Turbidity, Thymol Flocculation and Colloidal Gold Responses. NEEFE, J. R., BAHNSON, E. R., and REINHOLD, J. G. (1947). *Gastroenterology*, **9**, 656.

These three interesting papers deal with the now much-used turbidity or flocculation tests of hepatic function.

Shay and others consider particularly the thymol-turbidity test, but contrast it clinically with the cephalin-cholesterol and colloidal gold tests. They suggest a new modification of the thymol reaction—namely, a reading at 18 hours as well as at 30 minutes—and find that the "18-hour turbidity ratio" remains abnormal in infective hepatitis longer than do the other flocculation tests.

Mann and others have also used the thymol-turbidity test, and agree with previous workers that it is usually positive in infective hepatitis and negative in biliary obstruction. In other forms of hepatic disease the results are very variable. The authors' comment on the possible explanation of the flocculation is worth reading. They emphasize that the bromsulphthalein retention test is still the best indicator of injury to the liver when jaundice is absent.

Neefe and others found that the cephalin-cholesterol test provided evidence of hepatic disorder more often than did the other tests, but was for this reason of less value in distinguishing between intrahepatic forms of jaundice and extrahepatic biliary obstruction. They suggest that the thymol and gold tests are likely to have a similar mechanism, since their results run parallel and may differ considerably from those with cephalin-cholesterol. They conclude that a combination of all three tests is often useful in diagnosis, but if two only are chosen they should be the cephalin-cholesterol and thymol reactions.

(These papers support other workers' opinions of the value of these three tests of disturbed hepatic function, and will encourage research into their mechanism.)

J. W. McNee.

The Colloidal Scarlet Red Test Applied to Cerebro-spinal Fluid. (La reacción del rojo escarlata coloidal aplicada al liquido cefalorraquídeo.) TRIGUEROS, E. A., and REINLEIN, J. M. A. (1947). *Rev. clín. esp.*, **27**, 182.

Gray (*Arch. intern. Med.*, 1940, **65**, 523) suggested the use of the colloidal gold curve for testing hepatic function. Maizels proposed the substitution of the scarlet-red test instead of the colloidal gold test; this in turn led the authors of this paper to think that the scarlet-red test might be useful for replacing Lange's colloidal curve in examinations of the cerebrospinal fluids. They have examined 96 cases, and find that Lange curves and the curves obtained with scarlet red are for practical purposes identical. As the scarlet-red test is simpler and cheaper to carry out, the authors recommend it instead of Lange's gold test. *F. K. Kessell.*

Studies on the Mechanism of the Fanconi Syndrome. STOWERS, J. M., and DENT, C. E. (1947). *Quart. J. Med.*, **16**, 275.

The authors record a case in a man of 35 who had severe osteomalacia in the absence of any dietary deficiency of vitamin D, mild diabetes with renal glycosuria, amino-aciduria (without excessive cystine or methionine excretion), hypophosphataemia, and moderately impaired renal function; the liver showed focal centrilobular necrosis with nodular hyperplasia and a carcinomatous change (malignant hepatoma). Details of the investigations, including special metabolic studies of the amino-acid and sulphur metabolism and the ionic equilibrium of the serum and urine, are recorded. No significant change in the metabolism of sulphur-containing amino-acids was found. It is suggested that the excessive excretion of glucose and amino-acids is

dependent on the lack of adequate phosphate for their effective phosphorylation; thus their tubular reabsorption is impaired; besides a low renal threshold for phosphates, hyperphosphaturia may result from a chronic mild acidosis. *Henry Cohen.*

The Influence of Stilbamidine upon Kidney Function, Liver Function, and Peripheral Blood in Multiple Myeloma. ARAI, H., and SNAPPER, I. (1947). *N.Y. St. J. Med.*, **47**, 1867.

The authors discuss the toxic effects of stilbamidine, and report their findings in the treatment of 26 patients suffering from multiple myeloma. The kidney and liver functions were studied during and after stilbamidine therapy. In 24 patients there were no significant changes suggestive of impairment of kidney or liver function during or after stilbamidine therapy. Two patients who showed evidence of renal impairment during treatment were proved to have serious involvement of the kidney with myeloma. The authors conclude that although there is no conclusive evidence of renal or hepatic damage due to treatment with stilbamidine the influence on the kidney function of patients with myeloma must be carefully watched, especially in those with Bence-Jones proteinuria. *G. E. Hesketh.*

Diagnosis of Adrenal Tumours. A New Chemical Test. PATTERSON, J. (1947). *Lancet*, **2**, 580.

The differential diagnosis of adrenal tumour from adrenal hyperplasia is not aided by the estimation of the total urinary 17-ketosteroids, since they are increased in both conditions. One member of the group, however, dehydro*iso*androsterone, is increased in adrenocortical tumours in females. A new colour reaction is described for this substance. The reaction was positive in 3 cases of adrenal tumour with a very high daily ketosteroid output (215,335, and 1,980 mg.), negative in 6 cases of prepuberal virilism (output 24 to 81 mg.), and negative in 8 cases of secondary virilism (output 21 to 30 mg.). *H. Herxheimer.*

The Renal Regulation of Acid-base Balance in Man. I. The Nature of the Mechanism for Acidifying the Urine. PITTS, R. F., LOTSPEICH, W. D., SCHIESS, W. A., and AYER, J. L. (1948). *J. clin. Invest.*, **27**, 48.

The rate of excretion of titratable acid in the presence of acidosis with a concomitant infusion of large quantities of neutral sodium phosphate (*p*H 7.4) or creatinine was investigated. Eight experiments were performed on 4 healthy adult males. Acidosis was induced by the ingestion of 20 g. of ammonium chloride on the day preceding the observations. "Arterialized" venous blood was obtained from an arm vein for determinations of *p*H and CO_2 content. Glomerular filtration rate was determined by thiosulphate clearance; the plasma thiosulphate concentrations were maintained between 30 and 40 mg. per 100 ml. Simultaneous measurements of the rates of filtration, reabsorption, and excretion of monobasic and dibasic phosphate and carbonic acid showed that the quantity of titratable acid excreted was much greater than that which was filtered off in the glomerulus. When the plasma phosphate concentration reached 6 to 7 mM (millimols) per litre the hypothetical filtration-reabsorption mechanisms for phosphate and bicarbonate together could account for only one-third of the titratable acid excreted. Acid must have been added to the urine by the renal tubule. The rate of ammonia excretion averaged 0.066 mEq (milliequivalents) per minute which, though much increased over normal, was not high compared with that observed in diabetic acidosis. The ratio of ammonia to titratable acid, normally between 1 and 2.5, fell with the infusion of phosphate to 0.16 solely because of the increase in rate of excretion of titratable acid. In three experiments on 2 subjects the *p*H of the urine ranged between 4.48 and 4.6. Three experiments on 3 different subjects were made in which creatinine was substituted for phosphate. Again the carbonic-acid filtration could account for only 35 to 56% and the phosphate reabsorption for only 5.4 to 8.7% of the excreted acid. The mechanism for acid excretion in the human kidney is qualitatively similar and, judged by these experiments, quantitatively greater than that described in the dog kidney (Pitts and Alexander, *Amer. J. Physiol.*, 1945, **144**, 239). *C. C. N. Vass.*

The Renal Regulation of Acid-base Balance in Man. II. Factors Affecting the Excretion of Titratable Acid by the Normal Human Subject. SCHIESS, W. A., AYER, J. L., LOTSPEICH, W. D., and PITTS, R. F. (1948). *J. clin. Invest.*, **27**, 57.

This paper records a study on one subject (R.F.P.) but the results obtained were confirmed on other normal adult males. With the subject in a state of moderate 24-hour acidosis (plasma bicarbonate concentration 14.4 mM (millimols) per litre, plasma *p*H 7.37) the amount of phosphate in millimols excreted per minute during infusion of neutral phosphate bore a direct relation to the amount of titratable acid in milliequivalents excreted per minute. The results are in accord with the view that the urine is acidified by the exchange of hydrogen ions formed within the tubular cells for ions of fixed base in the tubular urine. *C. C. N. Vass.*

A New Clearance Substance in Renal Function Tests. (Eine neue Clearance-Substanz zur Nierenfunktionsprüfung.) FUHRMANN, G., and SCHUBERT, H. (1947). *Z. ges. inn. Med.*, **2**, 451.

The use of inulin and of creatinine clearance substances in testing renal function is unsatisfactory. A new agent, triticin, has been employed; it is also a fructose-yielding polysaccharide but of low molecular weight and soluble in water (25%). It may be injected rapidly and never causes shock. It is excreted completely in the urine in about 4 hours, none being retained by the tissues. Hydrolysis and the Selivanoff test, employed quantitatively with photometric measurement, proved satisfactory for estimation of triticin. The normal clearance values as well as the abnormal are approximately those obtained with inulin. *D. T. Barry.*

The Titratable Acidity, *p*H, Ammonia and Phosphates in the Urines of Very Young Infants. MCCANCE, R. A., and VON FINCK, M. A. (1947). *Arch. Dis. Childh.*, **22**, 200.

For some time after birth the kidney function is still immature, and it has been shown that the glomerular filtration rate, and the urea, sodium, and chloride clearances are low in newborn babies. This paper extends these observations by furnishing data on the *p*H, the titratable acidity, the ammonia coefficients, and the phosphate in the urine of newborn babies born in occupied Germany. Comparisons are made with specimens obtained from healthy Britains and Germans.

The data are based on the examination of urine from 72 infants, 36 adults, and 10 diabetics. All the babies were breast-fed and no additional water was given. The

following results were obtained: (1) The average *p*H tended to fall slightly after birth with an increase as milk flow was established. The *p*H did not differ from the average in urine from healthy adults. (2) The titratable acidity varied with the concentration of the urine and fell as the *p*H rose. (3) Ammonia coefficients were unaffected by the age of the baby but on the average were higher than in normal adults. (4) The ratio of the ammonia to the acidity was higher in infants than in adults. (5) Urine of newborn infants contained very little phosphate. This might lead to an incapacity to deal with an acidosis, should one occur. (6) Urine passed *in utero* was very dilute but differed in no other way from that passed in the first few days of life.
A. G. Watkins.

Response of New-born Children to Hypertonic Solutions of Sodium Chloride and of Urea. DEAN, R. F. A., and MCCANCE, R. A. (1947). *Nature, Lond.*, **160**, 904.

The authors examined the diuresis following injection of hypertonic saline and urea in infants suffering from meningomyelocele, and compared the findings with those in normal adults. While a diuresis occurred in both infants and adults, this was much less marked on a basis of surface area in infants, while after saline the osmotic pressure of the urine behaved differently in the two groups, falling in adults and rising in infants. Excretion of administered salt was much slower in infants. Similar results were obtained with urea, though the discrepancies between age groups were less marked and the diuresis less.
Alex Comfort.

Influence of the Diet on Urea Clearance. (Kostens Indflydelse paa Urinstof-clearance.) BANG, H. O., and NIELSEN, A. L. (1947). *Nord. Med.*, **36**, 2376.

The authors report the effects of alternate high-protein and low-protein diets on urea clearance in 5 nurses and 5 patients with healthy kidneys. The good diet was the full Danish hospital diet with 100 g. of meat added daily; the poor diet contained 25 to 30 g. of protein daily and had a calorie value of about 2,000. Subjects were given the diets for from 6 days to over 3 weeks and were in nitrogen balance; the nurses lost some weight when on the poor diet.

Urea clearance was estimated during two 1-hour or three ½-hour periods with an indwelling catheter. Diuresis was maintained to give maximal clearances. In all there were 25 estimations after a good diet and 15 after a poor one; with insignificant exceptions, individuals on the poor diets showed a fall in clearance down to as low as 44% of the previous values; the average fall was to about 70%. On return to the good diet clearance rose again. Other features of the poor-diet period were lowered urinary output (average 700 against 950 ml. a day), low plasma-urea levels (average 19 against 28 mg. per 100 ml.) and a low nitrogen output (average 4.1 against 10.7 g. a day).
A. M. M. Wilson.

Examination of Diodrast Clearance and Tubular Excretory Capacity in Man by Means of Two Single Injections of Diodrast (Umbradil). (In English.) JOSEPHSON, B. (1947). *Acta med. scand.*, **128**, 515.

A method of testing renal function is described based on changes of the plasma and urine concentration of "diodrast" after one intraglutaeal and one intravenous injection, combined with a clearance test of creatinine and inulin.

Nitrogen and Fluid Balance in Treatment of Acute Uremia by Peritoneal Lavage. Analysis of Peritoneal Washings for Protein, Nonprotein Nitrogen and Phosphorus. BASSETT, S. H., BROWN, H. R., KEUTMANN, E. H., HOLLER, J., ALSTINE, H. E. V., MOCEJUNAS, O., and SCHANTZ, H. (1947). *Arch. intern. Med.*, **80**, 616.

A woman aged 21, who in the course of progressive subacute glomerulo-nephritis became uraemic and practically anuric, was treated during the last 21 days of her life by peritoneal lavage. Altogether 257 g. of nitrogen was removed in the washing (140 g. of non-protein nitrogen and 117 g. of protein nitrogen), in addition to 92 g. in the gastric contents obtained by suction and 5 g. in the urine. At first substantial reduction in the non-protein nitrogen in the blood and inorganic phosphorus in the serum were obtained, but as the patient lost oedema fluid the values finally exceeded the prelavage figures.
L. H. Worth.

The Urinary Excretion of Amino Acids by a Cystinuric Subject. YEH, H. L., FRANKL, W., DUNN, M. S., PARKER, P., HUGHES, B., and GYÖRGY, P. (1947). *Amer. J. med. Sci.*, **214**, 507.

In 7 normal subjects the amino-acid excretion showed a remarkably uniform pattern, regardless of age differences. In a cystinuric child there were marked changes from the normal in the excretion of some amino-acids, both in absolute and relative amounts. Ingestion of methionine increased the urinary excretion of cystine in the cystinuric patient. It appears that in the normal subject arginine, cystine, and histidine, and in the cystinuric patient arginine, cystine, and lysine are present mainly in the unconjugated state, since the values for these amino-acids were essentially the same before and after hydrolysis of the urine.
L. H. Worth.

Chemical Studies in Children with the Nephrotic Syndrome. GOTTFRIED, S. P., STEINMAN, J. F., and KRAMER, B. (1947). *Amer. J. Dis. Child.*, **74**, 283.

For 2 years the authors studied 10 children between 16 months and 5 years old; 2 of the children died during this period. Extensive examinations were made of the blood chemistry and hepatic function. An increase or decrease of oedema seemed dependent on the serum protein and serum albumin being below or above 4 g. and 1.5 g. per 100 ml. respectively. Therapeutically, concentrated plasma and various forms of protein hydrolysate failed to produce any constant effect upon the total protein and serum-albumin levels. Some impairment of hepatic function was noticeable in all cases, and a defective production of protein is postulated. Low calcium values in serum were consistently present, and slight generalized osteoporosis was visible in radiographs.
W. G. Wyllie.

The Addis Count in the Prognosis of Acute Nephritis in Childhood. GILES, M. D. (1947). *Arch. Dis. Childh.*, **22**, 232.

The Addis count was used as a guide to prognosis in acute haemorrhagic nephritis. A red cell count of 600,000 per c.mm. or under in an 18-hour specimen of urine was considered to be normal. The result of the Addis count in 218 cases of acute haemorrhagic nephritis was compared with results of renal function tests, including estimations of non-protein nitrogen, serum proteins, and urea concentration and clearance tests. If the Addis count returns to normal and remains so it may be assumed that the renal lesion is healed and that a relapse is unlikely.
A. G. Watkins.

Comparative Absorption, Excretion, and Storage of Oily and Aqueous Preparations of Vitamin A. LEWIS, J. M., BODANSKY, O., BIRMINGHAM, J., and COHLAN, S. Q. (1947). *J. Pediat.*, **31**, 496.

Previous observations by these authors indicated that aqueous solutions of vitamin A are much better absorbed by premature infants than oily preparations, and the present investigation was undertaken "to ascertain whether the same phenomenon obtained for full-term infants, children, and adults." It was found that the average maximum blood level of vitamin A was five times higher after the aqueous product than after the oily solution. That this was due almost entirely to better absorption was shown by the fact that an average of 38% was wasted in the stools after ingestion of the oily product, whereas only 7% of the vitamin-A intake was lost in the stools when the aqueous solution was given. Similar studies on an 8-year-old boy with cystic fibrosis of the pancreas suggest that it should be possible to eliminate vitamin-A deficiency as a complication of this disease. It should also be possible to improve the absorption and effectiveness of vitamin D in the prevention and treatment of rickets. *M. Baber.*

Thiamine, Riboflavin, Nicotinic Acid, Pantothenic Acid and Biotin in the Urine of Newborn Infants. HAMIL, B. M., CORYELL, N., RODERUCK, C., KAUCHER, M., MOYER, E. Z., HARRIS, M. E., and WILLIAMS, H. H. (1947). *Amer. J. Dis. Child.*, **74**, 434.

Estimations of the daily urinary excretion of the vitamins of the B complex was undertaken in the newborn as a preliminary to assessing the nutritional status of the babies as regards these substances. Maximal average concentrations of thiamine and of pantothenic acid were found in urine excreted on the third day of life. For riboflavin the maximal average value was found on the first day, and for nicotinic acid and biotin on the second. The concentration of all vitamins in the urine decreased greatly after the first few days, very low levels being reached by the fifth day post partum in spite of abundant intake of breast milk. This large excretion of vitamins presumably indicates a high foetal storage. *M. Baber.*

The Effect of Dietary Restriction of B-complex Vitamins and Protein on the Excretion of Creatinine by Human Subjects. FRIEDEMANN, T. E., KINNEY, V. M., BERRYMAN, G. H., HENDERSON, C. R., and YOUMANS, J. B. (1948). *J. Nutrit.*, **35**, 117.

Seven men, aged 22 to 27 years, initially physically fit, were given 3 different diets and physical exercises over a period of 50 weeks, during which the variations in creatinine excretion and their relation to changes in weight and physical performance were studied. The results support the theory of early physiologists that there is a "constancy" in the relation between the creatinine excretion and the physical and nutritional state of the individual.

Vitamin C in the Blood and Urine of the Newborn and in the Cord and Maternal Blood. HAMIL, B. M., MUNKS, B., MOYER, E. Z., KAUCHER, M., and WILLIAMS, H. H. (1947). *Amer. J. Dis. Child.*, **74**, 417.

The plasma concentration of reduced ascorbic acid was estimated in samples of cord blood, capillary blood from 24 male breast-fed infants, and venous blood from their mothers, and compared with the amounts of vitamin C excreted in the infant's urine during the first week of life. The results showed wide variations, but cord blood contained by far the highest concentration of vitamin C, and the average value for the babies' blood, though only about half that for cord blood, was higher than for mothers'. Concentrations of vitamin C in urine were high during the first 2 days of life, but if the babies were not supplied with vitamin C urinary excretion dropped rapidly to low levels or disappeared as the blood level of the vitamin decreased. *M. Baber.*

Changes in Serum Calcium and Inorganic Blood Phosphorus after Treatment with Massive Doses of Vitamin D_2 in Some Cases of Tuberculosis in Children. (Variazioni del calcio sierico e del fosforo inorganico ematico dopo urtoterapia D_2 in alcuni casi di T.B.C. infantile.) RAGAZZIKI, F. (1947). *Riv. Clin. pediat.*, **45**, 473.

The behaviour of blood calcium and inorganic phosphorus levels was studied in 14 children with tuberculosis and 1 normal control; all were given massive doses of vitamin D_2 (calciferol), with and without intravenous calcium.

Cases were divided into 3 groups of 5. Patients in the first 5 were given 600,000 units intramuscularly and the serum calcium was estimated 1½, 3, 6, 24, 27, 33, 48, 51, 57, 72, and 96 hours later. There was a maximum rise of calcium late on the second or early on the third day to 0.8–1.9 mg. per ml. above the "resting" value: this maximum was maintained for 12 to 24 hours, and thereafter the level slowly fell, but in no case did it reach the former "resting" figure within 4 days. There was a small initial drop in blood calcium at 1½ hours. The second group was investigated over a period of 2 weeks, with daily serum calcium estimations for the first 7 days, and then on alternate days. A maximum rise on the third day was confirmed, and some cases showed a second but lesser peak about the sixth to eighth day. The blood calcium returned to its former level during the second week, in all cases by the fifteenth day. In the third group were 4 children with some form of tuberculosis, and 1 control. Phosphorus levels were found to parallel closely those of calcium in the serum, but the rise was more persistent. The daily intravenous injection of 10 ml. of 10% calcium gluconate produced greatly increased serum calcium, and the rise was more prolonged but there was no further effect on the phosphorus level. If calcium was given alone without vitamin D_2, there was no significant change in either the calcium or the phosphorus level, but if the vitamin was then administered there was an immediate and marked rise in calcium. Calcium given a week after the injection of calciferol had no effect in raising the calcium level in the blood. The normal control showed no change in phosphorus levels in spite of increase in serum calcium. The author recommends simultaneous administration of calcium and vitamin D_2, as calcium is then better utilized and the initial hypocalcaemic effect is obviated. *F. G. Sita-Lumsden.*

Utilization of Parenteral Protein Hydrolysate in the Normal. BARBORKA, C. J., CARROLL, W. W., and HEPLER, O. E. (1947). *Gastroenterology*, **9**, 579.

The preparation used in this study was "aminosol-fibrin," a partial acid hydrolysate of fibrin, 5% w/v, with 5% dextrose. About one-third of the amino-acids was in free form, and the remainder approximately in the tripeptide state. There were no toxic reactions, provided the solution was administered at a rate not

exceeding 80 drops a minute. Ten healthy 75-kg. medical students were chosen for the experiment. The administration of hydrolysate was preceded by a control period when the students were subjected to a protein intake varying between 75 and 10 g. daily. On this dietary a slight to definite negative nitrogen balance existed in all the subjects. A significant positive balance was obtained by the daily administration of 2 litres of the hydrolysate in all except those subjects whose diet contained only 10 g. of protein, these required 3 litres. The administration of larger doses was found to be unnecessary and wasteful. The positive balance was obtained only while the hydrolysate was being administered; and was rapidly lost during the ensuing 24 hours.

During administration there was no elevation of the non-protein nitrogen content of the blood and no significant alteration in the serum proteins.

J. B. Hannah.

The Effect of Dietary Fat upon Gastric Evacuation in Normal Subjects. ANNEGERS, J. H., and IVY, A. C. (1947). *Amer. J. Physiol.*, **150**, 461.

The authors, investigating the statement that fat delays gastric emptying, administered standard meals containing varying proportions of fat, and studied the emptying of the stomach by radiography. They conclude that while there is no difference in effect between saturated and unsaturated fats, statistically significant delay occurs in most subjects receiving a high-fat meal.

Alex. Comfort.

Anomalies of Intestinal Absorption of Fat. II. The Haematology of Idiopathic Steatorrhoea. COOKE, W. T., FRAZER, A. C., PEENEY, A. L. P., SAMMONS, H. G., and THOMPSON, M. D. (1948). *Quart. J. Med.*, **27**, 9.

It is interesting to have fresh confirmation that the pale faeces of steatorrhoea contain normal or increased amounts of bile pigments. The authors are inclined to attribute any increased pigment excretion to increased haemolysis rather than to a failure of absorption.

L. P. R. Fourman.

The Chylomicron Count in Normal Subjects and Patients with Sprue. FOURMAN, L. P. R. (1948). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 537.

The difficulties involved in counting chylomicrons are discussed, but the general conclusion is that counts of the fatty particles in serum do give some indication of the amount of absorbed fat in the serum at the time of the count. The chylomicron count does not, however, bear a close relation either to total fat absorption or to any individual fraction, though of these it is most nearly related to the neutral fat fraction. In patients with sprue depressed chylomicron curves were common, although the degree of depression was little related to the severity of the fat absorption defect as judged by results of fat-balance studies; moreover, treatment with liver improved the chylomicron count without greatly affecting the steatorrhoea. The author's deduction is that the proportion of absorbed fat which contributes to the chylomicron count is small. On the other hand, Elkes Frazer, and Stewart have shown that "chylomicron fat" represents an appreciable portion of the serum fats during fat absorption. To resolve this difficulty Fourman suggests that chylomicrons are removed from the blood more slowly than are other forms of absorbed fat. The general trend of results supports Frazer's partition hypothesis of fat absorption; and they are consistent with Stannus's suggestion that absorption of neutral fat in sprue is less affected than is absorption of split fat.

D. A. K. Black.

Aetiology of Steatorrhoea. FRAZER, A. C. (1947). *Brit. med. J.*, **2**, 641.

The author emphasizes the importance of balance experiments rather than estimations of the percentage of fat in the faeces in any study of fat absorption. In a diet containing 50 g. of fat 95% or more is normally absorbed. Defective emulsification was found in biliary obstruction and hepatic cirrhosis and in cases of atrophic pancreatitis, but in no other cases of defective fat absorption. In sprue systemic lipaemia is absent, though intraluminar changes are normal and 60 to 70% fat is absorbed: in regional ileitis, on the other hand, lipaemia is present. Adrenalectomy prevents the characteristic loading of the cell with fat globules, and this may be corrected by adequate salt therapy. Adrenal cortical deficiency has been suggested as a factor in the sprue syndrome, but if symptoms suggestive of adrenal insufficiency, such as dehydration and low serum-sodium levels, are relieved by appropriate treatment the fat absorption defect remains unchanged.

Alternative routes are available for fat absorption, the triglycerides largely absorbed in the particulate form passing by the lymphatic route, the fatty acids by the portal vein. Variations in the proportion of fat passing by these two routes may occur without any great effect on the total fat absorbed. Thus in lymphatic obstruction the quantitative decrease in fat absorption is much less than might be expected.

Since active absorption of fat occurs from the mid-duodenum downward the long-chain triglycerides are absorbed mainly in the particulate form in the upper intestine and as fatty acid in the lower intestine. The tolerance of patients with fat-absorption defects to different dietary fats appears to vary considerably. The *p*H of the upper intestine may also affect the routes of absorption, since an alteration towards increased alkalinity would produce a decrease in particulate and an increase in fatty-acid absorption.

O. L. V. de Wesselon.

The Diagnosis of Pancreatic Disease by Enzyme Tests. MORRISON, L. M. (1947). *J. Lab. clin. Med.*, **32**, 1107.

Specimens of duodenal contents were examined for proteinase, amylase, and lipase in fasting subjects and after the introduction of olive oil. In 6 cases of pancreatic carcinoma or cirrhosis these enzymes were consistently absent or were present only in traces. In 8 normal subjects the enzymes were occasionally but never repeatedly absent. In patients with non-organic digestive disturbances, or organic disease of the upper gastro-intestinal tract, the findings were not diagnostic. In 10 patients with "chronic low-grade or moderate inflammation of the pancreas as revealed at operation" the enzyme content was not abnormal.

Marjorie Le Vay.

Antithyroid Activity of Ergothioneine in Man. ASTWOOD, E. B., and STANLEY, M. M. (1947). *Lancet*, **2**, 905.

The previous findings that ergothioneine has an antithyroid effect similar to that of thiouracil, and that this substance occurs naturally in the blood have suggested that it might be involved in the regulation of thyroid

function. The substance, therefore, was administered to 5 normal persons in doses of 50, 200, and 400 mg. by mouth and 120 and 400 mg. intravenously, but without significant results.

Periodic Paralysis in a Patient with Exophthalmic Goiter Controlled by 6-Propylthiouracil. SEED, L. (1947). *W.J. Surg. Obstet. Gynec.*, **55**, 640.

The author's patient was a young man who developed symptoms of exophthalmic goitre and periodic paralysis after the aircraft carrier in which he was surving had been sunk. His serum potassium level was not significantly lowered, and administration of 2 g. of potassium chloride four times a day had no effect on his condition. His symptoms were relieved by treatment with 6-propylthiouracil, and his condition was finally controlled by 50 mg. three times a day. *J. B. Hannah.*

Metabolic Studies in Periodic Paralysis. (Stoffwechseluntersuchungen bei paroxysmaler Lähmung.) JANTZ, H. (1947). *Nervenarzt*, **18**, 360.

Nine patients (7 men, 2 women) suffering from periodic paralysis were studied in 8 years; altogether 68 attacks were observed. In every case the potassium content of the blood serum was lowered during an attack. The lowest value was 5.5 mg. per 100 ml. As soon as the paralysis receded, the potassium content of the blood serum reached the original level of about 20 mg. per 100 ml. Potassium chloride in 15- to 20-g. doses dissolved in water and given by mouth stopped every attack.

F. K. Kessel.

Potassium-calcium Metabolism in Chilblains. (Der Kalium-Calcium-Stoffwechsel bei der Perniosis.) SCHNEIDER, W., and ERASMY, H. (1947). *Arch. Derm. Syph., Wien.*, **186**, 137.

The normal ratio of potassium to calcium in the serum is taken as being 2 to 1. Twenty-three cases of chilblains were investigated and it was found that the average K/Ca ratio was 1:6; most of the values were below 2. The calcium was both relatively and absolutely increased; the potassium was absolutely increased but relatively diminished. The authors claim that in perniosis the preponderance of calcium in the blood varies with the arteriolar spasm, the latter being due to a low preponderance of sympathetic tone. It is not only a matter of the preponderance of one of the components; a failure of co-ordination is involved. It seems probable that perniosis is not due principally to the influence of cold but that is a disorder of the vegetative nervous system-hormone–electrolyte complex. *F. F. Jacobson.*

The Relative Importance of Dietary Sodium Chloride and Water Intake in Cardiac Edema. GORHAM, L. W., LESTER, D. E., WOLF, A. V., and SHULTZ, H. H. (1947). *Ann. intern. Med.*, **27**, 575.

The authors investigated 22 patients with cardiac oedema who were placed on a low-salt diet containing less than 1 g. of sodium chloride daily, and also 8 patients who were given a diet containing 3 g. of sodium chloride. Daily fluid intake varied from 1,000 ml. to 7,500 ml. Sodium and chloride levels were determined in serum twice weekly and a 24-hour specimen of urine was also collected daily. The loss of sodium on the diet of less than 1 g. of sodium chloride was shown to be appreciably greater than on the diet of 3 g. Very satisfactory clinical improvement was recorded in the majority of those patients on a diet containing less than 1 g. of sodium chloride. Some patients showed a urinary output exceeding the fluid intake. In general the clinical response of the patient appeared to depend on the ability to excrete sodium. In no case was there an adverse effect from the ingestion of large amounts of water when the diet was kept low in sodium chloride.

The object of the treatment is to maintain a low ratio of sodium intake to water intake, rather than a low sodium or a high water intake, and the restriction of salt is more effective in relieving cardiac oedema than is the forcing of fluid to a high level. *G. Hesketh.*

An Evaluation of Methods for Serum Proteins. BERRY, T. J., and PERKINS, E. (1947). *Amer. J. clin. Path.*, **17**, 847.

The serum protein concentrate was estimated in 327 consecutive blood donors, comparing the tubridimetric, the micro-kjeldahl, and specific gravity methods. The range of serum-protein concentrations was from 5.2 to 7.7 g., with a mean of 6.33 g. and a standard deviation of 0.58 g. It is concluded that the turbidimetric method (Looney and Walsh, *J. biol. Chem.*, 1939, **130**, 635) is the most suitable for use in clinical laboratories.

R. B. Lucas.

HAEMATOLOGY

A Simple Quantitative Formol-gel Reaction and its Relation to the Euglobulin and Gamma-globulin Content of Serum. (Een eenvoudige quantitatieve formolgelreactie en haar verband met het euglobulinegehalte en gamma-globulinegehalte van het serum.) VERHAGEN, B. A. (1947). *Ned. Tijdschr. Geneesk.*, **4**, 3524.

Gel formation occurs after the addition of a calcium-formalin solution to serum if the γ-globulin content is raised above a certain critical level. A simple quantitative technique is described to estimate the amount of γ-globulin present. The results compare well with those given by electrophoresis.

A Study of 114 Patients with Haemorrhagic Syndromes Seen within One Year. (A Critical Study of Tests of Haemostasis.) (Étude de 114 cas de syndromes hémorragiques examinés en l'espace d'un an. (Étude critique des épreuves fonctionnelles de l'hémostase.) TZANCK, A., and SOULIER, J. P. (1947). *Rev. Hémat.*, **2**, 429.

This long paper should be read by all interested in the haemorrhagic diatheses.

Thrombocytic Acroangiothrombosis. FITZGERALD, P. J., AUERBACH, O., and FRAME, E. (1947). *Blood*, **2**, 519.
Thrombocytic Thrombocytopenic Purpura. SINGER, K., BORNSTEIN, F. P., and WILE, S. A. (1947). *Blood*, **2**, 542.

These two papers refer to a rare fatal haemorrhagic state of acute or subacute course and unknown causation. The essential pathology is blockage of capillaries and arterioles by thrombi, mostly composed of platelets. Many organs are involved, and the clinical picture varies. The brain is often affected, and there may be mental symptoms. The spleen is sometimes palpable. A tendency to bleed is always present at some stage, and the platelet count is generally reduced.

Diagnosis of Thrombocytopenic Purpura. (Diagnostic des purpuras thrombopéniques.) DREYFUS, B., and SOULIER, J. P. (1947). *Rev. Hémat.*, **2**, 305.

In 9 patients with primary thrombocytopenic purpura the total number of megakaryocytes in the bone marrow varied from 450 to 4,000 per million nucleated cells, compared with counts of 175 to 600 in 5 normal subjects and in 3 patients with constitutional athrombopenic purpura. There was less evidence of platelet formation by budding, and an increase in young forms of megakaryocytes in the cases with primary thrombocytopenia. In 6 patients with secondary thrombocytopenia the megakaryocytes were present in normal or reduced numbers.

Clotting Defect in Hemophilia : Deficiency in a Plasma Factor Required for Platelet Utilization. BRINKHOUS, K. M. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 117.

Experiments are described in which platelet-free and platelet-rich plasmas were obtained from haemophiliacs and normals. Methylchlorosilane was employed to impart a non-wettable surface to all apparatus used so as to prevent the plasma from clotting during centrifugation. The author's results indicate that a plasma factor is required in addition to platelets for the proper coagulation of blood. This plasma factor is deficient in haemophilia.

Differences During Dicoumarol Therapy in the Quick and Russell Viper Venom Methods for Prothrombin Determination. WILSON, S. J. (1947). *Proc. Soc. exp. Biol., N.Y.*, **66**, 126.

Prothrombin estimations using both Russell viper venom and rabbit brain as sources of thrombokinase were carried out in parallel on 7 patients receiving dicoumarol therapy. The author concludes that the changes observed when rabbit brain was used reflect the clinical condition more closely, and that the use of the viper venom may be dangerous, as the prothrombin level so measured may appear to be a safe one when, in reality, the patient is about to bleed.

Hemophilia. A Report on the Mechanism of the Development and Action of an Anticoagulant in Two Cases. CRADDOCK, C. G., and LAWRENCE, J. S. (1947). *Blood*, **2**, 505.

Two haemophilic patients became refractory to treatment by whole blood transfusion or anti-haemophilic globulin. The patient's plasma contained an anticoagulant factor which was able to prolong the coagulation of normal plasma *in vitro*. The anticoagulant was of the nature of a γ-globulin and the authors suggest that this may have developed as an immune response to the many transfusions the patients had previously had.

Detection of Intravascular Clotting Tendency by Heparin Tolerances Principle. TUFT, H. S., and ROSENFIELD, R. E. (1947). *Amer. J. clin. Path.*, **17**, 862.

The authors have modified the Waugh-Ruddick test. They estimate the delay in the clotting of venous blood *in vitro* produced by adding to it heparin at a concentration of 0.01 mg. per ml. blood. In a group of 25 patients with various conditions of thrombosis or embolism the clotting times were 30 minutes or less, compared with 60 minutes using normal blood. It was shortened also in 15 out of 25 patients with myocardial infarcts.

A Modification of the Waugh-Ruddick Test for Increased Coagulability of the Blood and its Application to the Study of Postoperative Cases. SILVERMAN, S. B. (1948). *Blood*, **3**, 147.

Using a modified Waugh-Ruddick test (coagulation in the presence of a small standard concentration of heparin) in which recalcified plasma was used in place of whole blood, a postoperative increase in coagulability of the blood was demonstrated which began within 24 hours and lasted from 7 to 14 days after operation.

Prothrombin, Fibrinogen, and Protein Content, and Viscosity of the Blood in Thromboangiitis Obliterans. (Comportamento della protrombina, fibrinogeno, viscosimetria, e proteinemia nella tromboangioite obliterante di Burger.) SPOSITO, M., GIANNICO, O., and MARRAZZA, P. (1947). *Policlinico, sez. prat.*, **54**, 1235.

The authors studied 29 cases of Buerger's disease and claim that the prothrombin level was raised by an average of 15%. In 22 patients the fibrinogen averaged 570 mg. per 100 ml. The average total plasma protein was 8.1 g. per 100 ml. The authors believe that similar small rises in prothrombin and fibrinogen are found in many conditions where blood vessels are diseased. The use of dicoumarol is suggested.

Streakiness of Blood Films. Relation to Sedimentation-Rate and Plasma-Fibrinogen. BOVERI, R. M., WATERFIELD, R. L., and NEWMAN, T. H. (1947). *Lancet*, **2**, 831.

Streakiness in blood films spread on slides is due to an excess of plasma fibrinogen. This phenomenon is associated with a raised sedimentation rate, but not all bloods which undergo rapid rouleaux formation and have a raised sedimentation rate make streaky films. This is because a raised plasma globulin which increases rouleaux formation and rate of sedimentation does not cause streakiness.

The Action of Cobalt in Man. (Kobaltwirkungen am Menschen.) WEISSBECKER, L., and MAURER, R. (1947). *Klin. Wschr.*, **24/25**, 855.

This paper is a preliminary report on the action of cobalt on blood formation in man. Increases in reticulocytes and erythrocytes were noted in normal subjects and in anaemia due to haemorrhage and infection. The authors suggest that cobalt acts as a stimulus to erythropoiesis and indirectly affects the formation of haemoglobin. Cobalt is a toxic substance, and minor effects are described after the oral or intravenous administration of small doses.

Genuine Aplastic Anaemia with Complete Absence of Erythroblasts. (Über eine isolierte aplastische Anämie mit vollständigem Fehlen der Erythroblasten (Erythroblastophthise.)) BEGEMANN, H. (1947). *Klin. Wschr.*, **24/25**, 850.

The author describes the incidence in a man aged 59 of a rare disorder—primary aplastic anaemia affecting erythropoiesis only. The formation of leucocytes and platelets was unaffected.

The Development and Progression of Subacute Combined Degeneration of the Spinal Cord in Patients with Pernicious Anemia Treated with Synthetic Pteroylglutamic (Folic) Acid. ROSS, J. F., BELDING, H., and PAEGEL, B. L. (1948). *Blood*, **3**, 68.

The authors describe the development and progression of subacute combined degeneration of the cord in 11 of 25 patients maintained in satisfactory haematological remission with oral folic acid. The possibility that folic acid has actually a deleterious effect on the nervous system is discussed.

Changes in the Bone Marrow in Megaloblastic Anemias of Infancy Before and After Folic Acid Therapy. ZUELZER, W. W., NEWHALL, A., and HUTAFF, L. (1947). *J. Lab. clin. Med.*, **32**, 1217.

The authors studied the bone marrows of 15 children suffering from the megaloblastic anaemia of infancy. In some a primitive megaloblastic picture was seen, in others a less typical picture with cells intermediate in appearance between megaloblasts and normoblasts. In those cases with a typical megaloblastic marrow intermediate types were conspicuous for a day or two after folic-acid therapy and were then themselves replaced by normoblasts. This paper should be read by all interested in megaloblasts.

Refractory Megaloblastic Anemia. DAVIDSON, L. S. P. (1948). *Blood*, **3**, 107.

The author describes cases of anaemia with megaloblastic marrows partially or completely refractory to parenteral liver therapy. Some were examples of anaemia associated with sprue, pregnancy, or malnutrition. In 25 patients no cause was obvious. Ten of these responded slowly to numerous injections of liver extract and blood transfusions, the rest to proteolysed liver or folic acid. The superior value of oral as contrasted with parenteral liver in the treatment of refractory patients is discussed.

Experimental Study on the Localization of Castle's Intrinsic Factor in the Human Stomach. Antianemic Effect of Powdered Human Fundus and Pylorus. LANDBOE-CHRISTENSEN, E., and PLUM, C. M. (1948). *Amer. J. med. Sci.*, **215**, 17.

Extracts of the fundus of human stomachs were more effective in treating pernicious anaemia than were extracts of the pyloric region. In the case of the hog the reverse is true.

Acute Hemolytic Anemia in Primary Atypical Pneumonia Produced by Exposure and Chilling. COLMERS, R. A., and SNAVELY, J. G. (1947). *New Engl. J. Med.*, **237**, 505.

Anaemia rapidly developed in a woman with clinical and radiological signs of primary atypical pneumonia. A cold-agglutinin was present, titre 1: 1280 at 7° C., but still active (titre 1: 5) at 37° C. This was thought to be responsible for the anaemia.

A New Antibody in Serum of Patients with Acquired Hemolytic Anemia. STURGEON, P. (1947). *Science*, **106**, 293.

The authors claim to have demonstrated by means of the "indirect Coombs' Test" an antibody free in the sera of three patients with acquired haemolytic anaemia, a distinctly unusual finding. In one case the titre of antibody was 1: 4,000. The antibody could be eluted off the patient's red cells by incubating them at temperatures between 37° C. and 56° C.

The Cryptogenic Acquired Haemolytic Anaemias. FISHER, J. A. (1947). *Quart. J. Med.*, **16**, 245.

This is mainly a clinical review of 18 cases, in 4 of whom liver dysfunction was present. The results of splenectomy and of transfusions are considered.

Determination of Haemoglobin. II. The Haldane Haemoglobin Standard Compared with Iron and Gasometric Estimations. KING, E. J., GILCHRIST, M., WOOTON, I. D. P., DONALDSON, R., SISSON, R. B., MACFARLANE, R. G., JOPE, H. M., O'BRIEN, J. R. P., PETERSON, J. M., and STRANGEWAYS, D. H. (1947). *Lancet*, **2**, 789.

As a result of thorough large-scale investigations it has been found that the Haldane colour standard of British Standards Institution specification corresponds to 14.8 g. Hb. per 100 ml. of blood. If this figure is accepted, the supposed differences in the normal haemoglobin range between Britain and America as suggested by pre-war surveys can be explained. In reality, no differences probably exist.

Effect of β-Chlorethylamine Hydrochlorides in Leukaemia, Hodgkin's Disease, and Polycythaemia Vera. Report on Eighteen Cases. WILKINSON, J. F., and FLETCHER, F. (1947). *Lancet*, **2**, 540.

Eleven patients with chronic leukaemia, four with Hodgkin's disease, and three with polycythaemia vera were studied. There was a satisfactory reduction in the leucocyte level, splenomegaly, and lymph-adenopathy in the leukaemias, but in only 5 patients any rise in haemoglobin level. A striking improvement resulted in 3 of the 4 patients with Hodgkin's disease, but only 1 of the cases of polycythaemia showed any improvement. Except possibly in Hodgkin's disease, these dangerous and toxic compounds do not seem to have any advantage over *x*-ray therapy.

Familial Idiopathic Methaemoglobinaemia. Five Cases in One Family. GIBSON, Q. H., and HARRISON, D. C. (1947). *Lancet*, **2**, 941.

Quantitative estimations of methaemoglobin gave figures of 1.9 to 5.1 g. per 100 ml. blood in the five affected subjects. Treatment with methylene blue reduced these figures to 0.1 to 1.3 g. per 100 ml. It is thought that deficiency of coenzyme factor I is responsible for the presence of methaemoglobin, and that all the red cells contain a proportion of this pigment. Methylene blue is considered to catalyse the enzymic reduction of methaemoglobin.

The Relating O_2 in Bone Marrow Blood to Post-haemorrhagic Erythropoiesis. GRANT, W. C., and ROOT, W. S. (1947). *Amer. J. Physiol.*, **150**, 618.

Reduced oxygen tension has been held to be a stimulus for increased erythropoiesis. A single large haemorrhage resulted in a transient reduction in oxygen tension in the blood from the humerus of dogs, but repeated small haemorrhages were without effect. Both procedures were equally effective as stimuli of erythropoiesis. The authors experiments thus suggest that anoxia has no direct stimulatory action on the bone marrow.

Raynaud's Syndrome Originating from Reversible Precipitation of Protein. (In English.) HANSEN, P. F., and FABER, M. (1947). *Acta med. scand.*, **129**, 81.

The authors describe the onset of Raynaud's syndrome due apparently to the presence of an abnormal plasma euglobulin which was reversibly precipitated by cold. Multiple arterial embolisms were thus produced. The circulation could be restarted by applying warmth. The pathological diagnosis was aleukaemic plasma-cell leukaemia.

Some Investigations of Erythropoiesis in Human Bone-marrow Cultivated in Various Media. (In English.) PLUM, C. M. (1947). *Acta physiol. scand.*, **14**, 383.

This paper deals with the cultivation of aspirated normal and pathological bone marrow in Locke's solution with additions of serum, liver extract, folic acid, etc. It should be read by all interested.

Cold Hemagglutinins in Sickle-Cell Anemia. MCSWEENEY, J. E. J., MERMANN, A. C., and WAGLEY, P. F. (1947). *Amer. J. med. Sci.*, **214**, 542.

Sixty per cent of sera from 30 cases of sickle-cell anaemia contained cold agglutinins at a titre $> 1:40$; in 36% the titre was $> 1:80$. Three out of 30 control sera contained cold agglutinins of titre 1:40.

The Prozone Phenomenon in Rh Blocking Serums. HATTERSLEY, P. G., and FAWCETT, M. L. (1947). *Amer. J. clin. Path.*, **17**, 695.

A high degree of immunization is reported in 3 cases where a marked prozone prevented the recognition of hyperimmune or incomplete antibody by a single-tube technique, even when the test cells were suspended in 30% bovine albumin. Investigation of the causes of zone phenomena elicited the following facts. The zones were relatively unaffected by: (1) variation in cell concentration; (2) speed of centrifuging; (3) presence or absence of complement. They were influenced by: (1) temperature of incubation (prozone was most marked at 37° C., weak or absent at room temperature); (2) time of incubation prozone increased with prolonged incubation. The authors concluded that sera should never be heated, and that the bovine albumin test of Diamond and Denton (*J. Lab. clin. Med.*, 1945, **30**, 821) can be improved by centrifuging both immediately before and after incubation, the cell deposits being examined microscopically after each spinning. (Sera from cases with such suggestive histories should be examined by the indirect sensitization test of Coombs and others.) *John Murray.*

Studies on the Conglutination Reaction, with Special Reference to the Nature of Conglutinin. WIENER, A. S., HURST, J. G., and SONN-GORDON, E. B. (1947). *J. exp. Med.*, **86**, 267.

The authors have tested various blood-protein-containing preparations for their power of agglutinating Rh-positive cells which have absorbed incomplete Rh antibody. Albumin, fibrinogen, and β-globulin make up conglutinin, or *X*-protein. One part of albumin added to 5 parts of human plasma notably increases the conglutination activity of the latter.

The conglutinin titre of foetal blood is low, but quickly increases after birth. Wiener recommends removing part of the donor's plasma before exchange transfusions so as to reduce its conglutinin content.

Haemolytic Icterus (with Special Reference to its Pathogenesis). (Icterus haemolyticus. Med saerlig henblikk på patogenesen.) OWREN, P. A. (1947). *Tidsskr. norske Laegeforen*, **67**, 665.

The author reports interesting serial blood and bone marrow findings which suggest that in familial haemolytic icterus the "haemolytic crises" are due to temporary marrow aplasia rather than to increased haemolysis (see also *Blood*, 1948, **3**, 231). The mechanisms of erythrocyte destruction in the familial and acquired types of haemolytic anaemia are also considered and contrasted.

Erythropoiesis in Chronic Erythroblastosis in Adults. (L'erythropoïèse dans les erythroblastoses chroniques de l'adulte.) OLMER, J., and GASTAUT, H. (1947). *Ann. Méd.*, **48**, 458.

This interesting, obscure, and not very uncommon disorder is generally referred to in British and American literature as myelosclerosis or chronic non-leukaemic myelosis. Anaemia, erythroblastaemia with some primitive granulocytes, a greatly enlarged spleen, moderate enlargement of the liver, and an atrophic or fibrotic marrow are the main characteristics. Its course is a prolonged one.

Studies in Erythroblastosis Fetalis. I. Activation of the Incomplete Rh Antibody by the Blood Serum of Full-term and Premature Newborn Infants. WITEBSKY, E., RUBIN, M. I., and BLUM, L. (1947). *J. Lab. clin. Med.*, **32**, 1330.

Studies in Erythroblastosis Fetalis. II. Investigations on the Detection of Sensitization of the Red Blood Cells of Newborn Infants with Erythroblastosis Fetalis. WITEBSKY, E., RUBIN, M. I., ENGASSER, L. M., and BLUM, L. (1947). *J. Lab. clin. Med.*, **32**, 1339.

The authors have found that cord sera from newborn infants and in particular from premature infants are generally much less active than the sera of adults in causing agglutination of Rh-positive cells which have been subjected to "incomplete" or blocking Rh antibody. The sera of infants 24 to 48 hours old were more active than cord sera. It is suggested that this increase in serum activity developing after birth may be connected with the delayed post-natal onset of haemolysis sometimes observed in haemolytic disease of the newborn.

In the second paper the authors recommend suspending the cells suspected of sensitization in adult serum, preferably on a tile.

Breast-feeding in Erythroblastosis Foetalis. CATHIE, I. A. B. (1947). *Brit. med. J.*, **2**, 650.

The author has demonstrated that Rh antibody in serum is not destroyed by 1 hour's incubation in infant's gastric juice. However, there was no evidence of any absorption when breast milk containing antibodies or high-titre Rh anti-serum was fed to Rh-positive infants or to an adult. It is concluded that it is safe and justifiable to breast-feed infants with haemolytic disease of the newborn even if the mothers' milk contains Rh antibodies.

Multiple Myeloma. A Survey Based on Thirty-five Cases, Eighteen of which came to Autopsy. LICHTENSTEIN, L., and JAFFE, H. L. (1947). *Arch. Path., Chicago*, **44**, 207.

In this review clinical, biochemical, haematological, and pathological features are considered. In half the cases serum calcium is raised. Calcium granules are

deposited in the kidneys and renal failure may follow. Secondary hyperplasia of the parathyroids may develop, but was not seen in this series. Hyperproteinaemia, and more specifically hyperglobulinaemia, is seen in about half of the cases. Bence-Jones proteinuria was present in 10 of 26 cases; it is very variable in quantity and occurrence. The serum uric acid, as in leukaemia, is increased. Amyloid is mentioned as a complication in 10% of cases, and the disease is considered to be related to the leukaemias. The plasma cells, which vary in maturity, are thought to be abnormal neoplastic haematic cells.

MORBID ANATOMY AND HISTOLOGY

Cranial Trauma and Extrapyramidal Involvement: Cerebral Changes Simulating Those of Anoxia. A Clinico-pathologic Report of Three Cases. MALAMUD, N., and HAYMAKER, W. (1947). *J. Neuropath. exp. Neurol.*, **6**, 217.

Three cases of head injury are described in which the chief lesions were in the lenticular nuclei. In 1 case there was also softening of the substantia nigra and pseudo-laminar cortical necrosis, and most of the Purkinje cells of the cerebellum had disappeared. Cessation of respiration during anaesthesia for reduction of fractures may have been a causative factor in this case. Fat embolism was not found. The authors suggest that similar anoxic lesions may be the basis of cases of post-traumatic Parkinsonism. *J. G. Greenfield.*

The Use of the Smear Technique in the Rapid Histological Diagnosis of Tumors of the Central Nervous System. Description of a New Staining Method. MORRIS, A. A. (1947). *J. Neurosurg.*, **4**, 497.

The author reports favourably on the use of a special eosin-methylene-blue staining technique for the rapid diagnosis of cerebral tumours by the smear biopsy method. (For details of the technique, which is not his own, the author's original article should be consulted.) By this technique smears can be examined under the microscope within 30 to 40 seconds of being made. The author has used the technique in a series of 116 tumours of the central nervous system. He considers the method of great value, although it can, at best, give only a presumptive diagnosis, which should be confirmed whenever possible by routine histological techniques.
J. G. Greenfield.

Positive and Negative Aspects of Hypothalamic Disorders. (In English.) BROUWER, B. (1947). *Proc. Akad. Wet. Amst.*, **50**, 1038.

Ten cases with symptoms attributable to lesions of the hypothalamus were compared with experimental hypothalamic lesions. Discrepancies were found in all types of lesion. Three cases of diabetes insipidus showed bilateral destruction of the supra-optic nuclei as was expected from the experimental work, but one case with similar destruction did not have diabetes insipidus. All the other expected lesions are discussed.

Primitive Malignant Tumours of the Nose and Pharynx. (Primitive maligne Geschwülste der Nasenund Rachengegend.) LÜDIN, M. (1947). *Prat. oto-rhino-laryng., Basel*, **9**, 148.

The author discusses the literature on those tumours most often found in the nasopharynx or oropharynx which invade the lymph nodes early and produce distant metastases. Because of the frequent findings of an epithelial syncytium, in the meshes of which are embedded masses of rounded cells, these tumours have been described as carcino-sarcomata, lympho-epitheliomata, and reticulo-cell sarcomata. The prevailing tendency is to consider these embryonal tumours as sarcomata. Some resemble undifferentiated carcinomata, others lympho-sarcomata, and a third group are so primitive that no distinction between epethelial or mesenchymatous tissue is possible.

The clinical notes and detailed histology of 21 cases are given. The author considers that those which can with certainty be said to arise from epithelium may be called carcinomata, and those which can be said to arise from a mesenchymal matrix may be called sarcomata, but that the primitive undifferentiated tumours should be called "meristomata." *Stephen Suggit.*

The Histology of the Irradiated Larynx. PRICOLO, V., and PIZZETTI, F. (1947). *Radiolog. med., Torino*, **33**, 586.

The authors find that many of the vascular and cartilaginous changes previously attributed to irradiation of this region are to be found in normal specimens between the ages of 30 and 50. Comparison of controls with non-irradiated and irradiated carcinomatous larynges shows that the only effect attributable to *x*-rays is round-celled infiltration of the mucosa and sub-mucosa.

Primary Carcinoma of the Trachea. ELLMAN, P., and WHITTAKER, H. (1947). *Thorax*, **2**, 153.

The authors summarize and discuss the literature on tracheal tumours and describe a case shown at necropsy to be one of carcinoma of the trachea. The patient complained of hoarseness, largely due to recurrent laryngeal palsy, and had signs deriving from transient collapse of the lower lobe of the left lung. The tumour arose on the posterior wall of the trachea in its lower third. Part of the growth, nearly 2 cm. in diameter, projected into the lumen; a much larger mass, measuring some 5 × 3 cm., extended into the mediastinum. Paratracheal glands and those at the bifurcation of the trachea were involved by metastases, but no other spread of the tumour was found. Section of the growth showed it to be a squamous-celled carcinoma.
W. D. W. Brooks.

An Unusual Hamartoma (So-called Chondroma of the Lung). SIMON, M. A., and BALLON, H. C. (1947). *J. thorac. Surg.*, **16**, 379.

So-called chondromata of the lung are not pure cartilaginous tumours but contain mixtures of elements normally encountered in the bronchial wall and are therefore more properly referred to as hamartomata or hamartoma chondromatosum pulmonis. Such a tumour is usually considered benign and slow-growing. It may, however, undergo malignant change. The tumour here described is unusual because of its enormous size and the fact that it caused symptoms and radiologically simulated bronchiogenic carcinoma.
George A. Mason.

The Myoepithelium in Certain Tumours of the Breast. BIGGS, R. (1947). *J. Path. Bact.*, **59**, 437.

The author describes examples of various neoplastic and hyperplastic lesions of the human and canine breast in which she believes myoepithelial cells to be demonstrable.

Is the Brenner Tumour always a Benign Neoplasm? (Ist der Brenner-tumor immer eine gutartige Neubildung?) DUBRAUSZKY, V., and MASSENBACH, W. VON (1947). *Zbl. Gynäk.*, **69**, 370.

The authors believe that the Brenner tumour originates from the coelomic epithelium, and that some of the solid carcinomata and cystic adenocarcinomata of the ovary may originate in a Brenner tumour. They describe the tumour from a woman aged 70, in which the medullary part of the tumour, as distinguished from the cystic portions, showed definite signs of malignancy, and the transition from benign to malignant epithelium could be followed. The Brenner tumour does not produce any oestrogenic hormone, and bleeding in this case was caused by a polypoid hyperplasia of the endometrium. This would appear to be the first described case of a definitely malignant Brenner tumour.

Composition and Structure of the Liver Cell in Pregnancy. KOSTERLITZ, H. W., and CAMPBELL, R. M. (1947). *Nature, Lond.*, **150**, 676.

The desoxyribonucleic-acid content of the liver of pregnant rats was shown to be significantly increased by the third week of gestation and was greater than could be accounted for by the increase in maternal body weight. The total amount was related to the sum of maternal and foetal body weights and was independent of the diet. A specific rise of 40 to 45% in the ratio of ribonucleic to desoxyribonucleic acids also occurred; this was not due to dietary changes during pregnancy and was correlated with foetal body weight. The increased ribonucleic-acid content was particularly apparent in the periportal area. Glycogen content of the liver was much decreased.

J. Dawson.

Glycogen Formation and Deposition in the Human Liver. (Über Glykogenbildung und Glykogenablagerung in der menschlichen Leber.) EGER, W., and KLÄRNER, G. (1948). *Virchows Arch.*, **315**, 135.

Estimation of glycogen in the liver in 250 necropsies, 19 of them performed on subjects who had died suddenly ("normal cases"), and the rest on subjects who had died after illness of longer duration, was carried out by Pflüger's method. The values were calculated in terms of wet weight, dry weight, or "dry protein weight"—that is, dry weight minus fat and glycogen. The values for glycogen were higher in "normal cases," averaging 7.09% against 2.04% of dry weight in the other cases. No antagonism was found between the quantities of fat and glycogen. The results of histological estimation by the methods of Bauer and Best were not in accord with the chemical determinations. In "normal cases" the glycogen is located in the central parts of the lobules. Deposition in the periphery of the lobules and in the nuclei is explained as a sign of damaged function of the liver. It is suggested that glycogen is normally built up in the nucleus, then goes into the body of the cell for storage; this hypothesis would be in accordance with the fact that livers rich in glycogen contain more uric acid (3,232 mg. per 100 g. of dry protein weight against 1,437 mg.).

O. Neubauer.

Gaucher's Disease Without Splenomegaly. MORGANS, M. E. (1947). *Lancet*, **2**, 576.

A family affected by Gaucher's disease is described. In the father the diagnosis was first made when he was 67 years old; sternal puncture revealed Gaucher cells and radiographs showed translucent areas in bone. There was enlargement of the liver, but the spleen was not palpable. Two of his 3 children, a man aged 23 and a girl aged 14, had radiological evidence of the disease, and in the third child, a boy aged 12, the radiological appearances were not conclusive. The family history indicated that the disease may have occurred in the two previous generations. The series is remarkable in three ways: the absence of splenomegaly, the appearance of the disease in successive generations, and the survival of 1 patient in active life to a good age.

K. Black.

The Hepatitis of Hyperthermia. Report of a Fatal Case. BRAGDON, J. H. (1947). *New Engl. J. Med.*, **237**, 765.

A fatal case of hepatitis and hepato-renal syndrome is described in a patient suffering from sulphonamide-resistant gonorrhoea who was treated by artificial fever. The pathogenesis is discussed.

Gelatin Nephrosis. Renal Tissue Changes in Man Resulting from the Intravenous Administration of Gelatin. SKINSNES, O. K. (1947). *Surg. Gynec. Obstet.*, **85**, 563.

The author has studied the effects of gelatin given intravenously in 8% saline as a treatment for peripheral vascular collapse; 23 patients so treated came to necropsy, most of them having been treated surgically for malignant disease within the abdomen. The kidneys affected by gelatin were swollen and the cut surfaces bulged. Microscopically the kidneys were found to be the seat of a change similar to the nephrosis caused by sucrose. The most important single factor related to these changes was the time interval between gelatin administration and death. All patients examined within 67 hours of their last gelatin injection showed gelatin nephrosis; in patients who died more than 120 hours after the last gelatin injection these changes were not seen.

I. Aird.

Zinc Sulphate Flotation of Faeces. ELSDON-DEW, R. (1947). *Trans. R. Soc. trop. Med. Hyg.*, **41**, 213.

The zinc sulphate flotation technique for the concentration of ova and cysts in faeces (Faust *et al.*, 1938) is described.

In 1,539 specimens, mostly from cases of suspected amoebiasis, this technique was compared with a direct faecal film. A considerable increase in positive results was obtained with most intestinal parasites and protozoal cysts. Thus, with *Entamoeba histolytica, Bact. coli, E. nana*, and *Iodamoeba bütschlii*, the ratio $\frac{\text{percentage positive results with flotation technique}}{\text{percentage positive results with direct film}}$ was 175.5, 170.6, 315.2, and 577.8 respectively; with *Giardia lamblia* and *Cholomastix mesnili*, 500, and 300; with *Ascaris lumbricoides, Trichuris trichura*, and *Ancylostoma*, 122.8, 201.9, and 538.2. The ova of *Taenia* and *Schistosoma mansoni* did not float well, but could often be found in the sediment after the final spinning. This method should only be used, however, as an adjunct to the direct film examination, which reveals the trophozoites in addition.

J. L. Markson.

Thrombosis of the Hepatic Veins. The Budd-Chiari Syndrome. THOMPSON, R. B. (1947). *Arch. intern. Med.*, **80**, 602.

The author reviews 95 cases of thrombosis of the hepatic veins collected from the literature and reports a

further 2 cases. He suggests that the term "Budd-Chiari syndrome" should be reserved for cases in which there is evidence of gross blockage of the hepatic veins; cases without such evidence should be classified as "thrombosis of hepatic veins."

In both groups there were three main sites of obstruction: the hepatic veins, their ostia, and the inferior vena cava. Severe venous engorgement in the acute stage resulted in central lobular necrosis. Later cirrhotic changes and nodular hyperplasia were common. Portal thrombosis was rare and usually a terminal event. Venous engorgement, severe in the spleen and of varying degree in the intestines, was usually found. The age incidence varied from 17 months to 61 years, the average being 34. Males were affected more often than females. There were 8 cases of polycythaemia rubra vera and 4 pregnant women. A probable cause was phlebitis of the hepatic veins, which may be part of a more generalized vascular disease. In the full picture epigastric pain of variable severity was usually the initial symptom. Vomiting occurred in about a quarter of the cases and was severest at the onset. Ascites and marked hepatic enlargement were usual. The liver was generally tender. Splenic enlargement was much less frequent. The development of venous collaterals was one of the most important signs. Oedema of the legs developed in about half the cases. Jaundice was slight or latent. The author suggests that the diagnosis of minor thrombosis should be kept in mind in cases of obscure pain in the upper abdomen, especially where there is evidence of liver damage. As a rule the disease was of short duration but in a small group it lasted for 10 to 28 years. Of 9 patients who underwent operation, 8 died soon afterwards. *J. B. Mitchell.*

Pathologic Significance of the Ductus Arteriosus. Its Relation to the Process of Arteriosclerosis. BLUMENTHAL, L. S. (1947). *Arch. Path.*, **44**, 372.

The histological changes associated with closure of the ductus arteriosus range from replacement of the normal smooth muscle by fibrous tissue to formation of localized areas of necrosis. It is suggested that these changes are the result of anoxic conditions produced by the contraction of smooth muscle of the wall of the ductus. These changes are discussed in relation to the wider problem of arteriosclerosis and to the possibility that localized anoxaemia is the fundamental mechanism in both processes. (There is a useful bibliography.)
R. H. D. Short.

Cholesterol Crystal Embolism of Minor Organ Arteries and its Consequences. (Cholesterinkrystallembolie kleiner Organarterien und ihre Folgen.) MEYER, W. W. (1947). *Virchows Arch.*, **314**, 616.

A new form of embolism—embolic transport of cholesterol crystals from atheromatous ulcers of the aorta into small arteries of different organs—is described. The crystals are easily detected in the lumen of arteries of kidneys, brain, and meninges in frozen sections. Two cases are reported in detail. The embolized cholesterol crystals lead to a particular form of arteritis with numerous giant cells, and later to obliteration of the arteries, with subsequent changes in the tissues, such as scars on the surface of the kidneys and circumscribed softening of the brain. *O. Neubauer.*

Pulmonary Embolism by Amniotic Fluid. Report of 3 Cases with a New Diagnostic Procedure. GROSS, P., and BENZ, E. J. (1947). *Surg. Gynec. Obstet.*, **85**, 315.

Three new cases are presented of a rarely described form of embolism, due to amniotic fluid. Previously 12 cases have been described. In all cases the diagnosis has been made at necropsy, generally after histological examination.

Summary of the necropsy findings shows death to have been asphyxial without gross lesions. The diagnosis is made by finding emboli rich in polymorphonuclear leucocytes, mucin, bile-stained debris (meconium), epithelial squamae, lanugo hair, and granular debris with or without fatty elements in histological preparations of the arteries, arterioles, and capillaries of the lungs. Blood from the right heart or the inferior vena cava (which may be obtained when permission for necropsy is withheld) may show three strata (instead of two) after centrifugation. The presence of the third (top) layer is considered pathognomonic. The top layer is separated. After fixation in alcohol, Zenker's fluid, and formol, sections stained by mucicarmine and Mallory's phosphotungstic acid and haematoxylin are prepared. Constituents of meconium and amniotic fluid are demonstrable.

In these 3 cases (all in multiparae) severe post-partum (1 intra-partum) irreversible shock was followed by death within 65 minutes. No case could be classified as one of difficult labour. Routine aspiration of blood (see above) should be undertaken in all obstetric deaths. (Infant weights are not recorded.) *Magnus Haines.*

Primary Hypertrophy and Hyperplasia of the Parathyroid Glands as a Cause of Hyperparathyroidism. ROGERS, H. M., and KEATING, F. R. (1947). *Amer. J. Med.*, **3**, 384.

The authors review 22 cases from the literature and add 4 from the Mayo Clinic. On analysis there appears to be some curious connexion between hyperparathyroidism and duodenal ulcer; this lesion was present in 3 of the 4 cases here reported, and the authors have noted the co-existence of duodenal ulcer in approximately one-third of all the patients with hyperparathyroidism seen at the Mayo Clinic. They state that "this association seems too frequent to be coincidence but one can only conjecture as to its meaning."

The Intestinal Phase of Human Trichinosis. STRYKER, W. A. (1947). *Amer. J. Path.*, **23**, 819.

Living adult trichinae, including gravid females, were demonstrated in the intestine of a fatal case of human trichiniasis 54 days after ingestion of infected pork—the longest period of persistence of adult trichinae in the human intestine thus far reported. The possibility of continued release of larvae over an even longer period must be taken into account in the therapeutic management of trichiniasis.

Endemic (Murine) Typhus. Report of Autopsy Findings in Three Cases. BINFORD, C. H., and ECKER, H. D. (1947). *Amer. J. clin. Path.*, **17**, 797.

The authors give a detailed description of 3 fatal cases of endemic typhus. An acute interstitial myocarditis, with mononuclear and plasma-cell infiltration but with no apparent damage to the cardiac muscle fibres, was present in all, and was probably a major factor contributing to death. In 2 cases there was severe interstitial orchitis, and in 1 case skin lesions, closely resembling those found in epidemic typhus, showed a cellular perivascular infiltration with oedema and obliteration of several vessels by inflammatory cells or by thrombi. No typical typhus lesions were found in brain or lungs.

Comparative Morphological Investigations on the Mammary Glands of Mice of Different Origin. Investigations Concerning the Morphological Test of the So-Called Milk Factor. (In Russian.) POGOSYANTS, E. E. (1947). *Arkh. Patol.*, **9**, No. 2, 64.

Experiments were carried out to devise a morphological test for the so-called milk factor. Mammary glands were examined by the whole mount technique in in-bred strains of varying incidence of breast cancer and the results confirmed the findings of other workers on the differences in structure in mammary glands between high and low breast cancer strains manifested by greater alveolar proliferation with nodules on hyperplastic tissue. In addition, 35 low-incidence strains received the milk factor whilst 5 to 25 days old, and 10 of these developed breast tumours; 14 receiving the extract when 30 to 45 days old did not develop tumours. The author concludes that the morphological examination of mammary glands of mice by the whole mount technique is a satisfactory test for the presence of the milk factor in breeding females not less than 6 months old.

The Antigenic Relationship of Lymphogranuloma Venereum and Psittacosis by Skin Test in Humans. POLLARD, M., and WITKA, T. M. (1947). *Tex. Rep. Biol. Med.*, **5**, 288.

A psittacosis antigen suitable for carrying out skin tests was prepared, and tests were carried out with this antigen and with lygranum on patients with venereal disease in an army hospital. All 8 confirmed cases of lymphogranuloma venereum gave a severe reaction to the psittacosis antigen; 6 suspected cases, of which 2 were confirmed serologically, gave indefinite reactions; while 5 cases of penile ulcer, 8 cases of syphilis, and 3 suspected cases of syphilis were negative to both skin antigens. Lymphogranuloma venereum and psittacosis thus show an antigenic relation in the skin test, and this test is evidently not suitable for diagnosis when there is any possibility of the diseases co-existing. *D. J. Bauer.*